Assessing the lifespans of coldwater octopods (Cephalopoda: Octopodiformes)

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Summary

A general trend in ecology is that marine species living in cold-water, such as the poles and the deep sea, have extended lifespans compared to their relatives that inhabit temperate and tropical waters. Temperature influences the pace of life of ectotherm organisms. Species permanently living in low temperatures have life history traits that are associated with high longevity, including slow growth, late maturity and low fecundity. These traits were observed in various cold-water invertebrates that belong to echinoderms, crustaceans and molluscs and that produce low numbers of offspring and exhibit longer lifespans compared to their warm water counterparts. However, detailed information on life history traits is absent for the majority of deep-sea and polar species.

An important group of marine invertebrates that occurs in almost all marine ecosystems are the cephalopod molluscs (e.g., octopods, squids, and cuttlefishes). Among them, the superorder Octopodiformes (i.e. octopods and vampire squid) has representatives living in almost all latitudinal zones, from tide pools to abyssal depths. Octopodiform species from temperate and tropical regions (>10°C) typically grow fast, mature early and have a single reproductive event dying soon after (semelparity), which results in short lifespans rarely exceeding 2 years. Less is known about life histories of deep-sea and polar species. Deep-sea and polar incirrate octopods (unfinned species) are also semelparous, but they produce large eggs and brood them in cold-waters that may delay embryogenesis for significant periods of time. The vampire squid, which inhabits the deep sea have multiple reproductive cycles that may require several months per cycle to be completed. Although longevity is unknown for the majority of cold-water octopodiforms, their life history traits suggest considerably longer lifespans compared to other octopods which typically live for one year. This thesis dissertation investigates longevity of deep-sea and Antarctic octopodiform species using life history traits and the quantification of growth increments in the octopods' beaks (chitinous jaws).

The first chapter investigates how life history traits including embryonic development, hatchling size, size- and age-at-maturity are correlated with habitat characteristics (water temperature and depth of occurrence), using published data of 26 octopodiform species. For species without information on age, time to reach maturity was estimated using a model for embryonic development at low

temperatures. The results showed significant negative correlation between age at maturity and average habitat temperatures. The depth of occurrence positively correlated with time to reach maturity. While lifespans of temperate and tropical species last typically less than 1 year, octopods living in the Antarctic and deep seas were estimated to mature after 3 to 5 years. Although the results need direct validation, they are congruent with lifespan estimates of other invertebrate groups inhabiting polar and deep seas.

The second chapter tests the hypothesis that the incirrate benthic octopods living in the vicinity of the Antarctic Peninsula (-2 to 2°C) have longer lifespans than their temperate and tropical relatives. Age was investigated in specimens of the family Megaleledonidae (*Pareledone* spp., *Adelieledone* spp. and *Megaleledone setebos*) and in *Muusoctopus rigbyae*, using the quantification of growth increments deposited in their beaks. It was observed that specimens in advanced maturity stages of all species frequently had more than 400 increments in the beaks. The periodicity of increments deposition for these species is unknown. However, the beaks of females of *Pareledone charcoti* maintained alive in aquaria for 12 months had 219 to 364 growth increments, suggesting that increment deposition in this species takes longer than one day. Age estimates are discussed and were compared to those in other octopod species in which the periodicity of beak growth increments has been validated to be daily.

The third chapter also uses the quantification of growth increments in beaks to assess if the deep-sea pelagic octopodiforms *Japetella diaphana* and the vampire squid *Vampyroteuthis infernalis* have longer lifespans compared to benthic relatives. The maximum number of growth increments in beaks of *J. diaphana* and *V. infernalis* was 207 and 375, respectively. If daily deposition of the increments is assumed, the resulting lifespans are incongruent with the passive mode of life of these two species. Their remarkable low metabolic rates and reproductive biology rather suggest that growth increments in the beaks may require more than one day to be formed.

Zusammenfassung

Ein genereller ökologischer Trend ist, dass Tierarten, die in kalten Gewässern leben, wie z. B. an den Polen und in der Tiefsee, eine längere Lebensdauer haben als vergleichbare Arten im wärmeren Flachwasser und in tropischen Gewässern. Die Temperatur reguliert den Lebensrhythmus von ektothermen Organismen. Arten die dauerhaft bei niedrigen Temperaturen leben, zeigen Lebensmerkmale, die auf eine lange Lebensdauer deuten, wie langsames Wachstum, späte Geschlechtsreife und geringe Fruchtbarkeit. Diese Merkmale wurden bei einigen wirbellosen Kaltwasserformen aus den Gruppen der Stachelhäuter, Krebstiere und Weichtiere beobachtet, die weniger Nachkommen produzieren, aber eine längere Lebensdauer aufwiesen als verwandte Arten aus wärmeren Regionen. Für die meisten Tiefsee- und Polararten fehlen jedoch detaillierte Angaben zu den charakteristischen Lebensmerkmalen.

Eine wichtige Gruppe der Wirbellosen, die in fast allen Meeresökosystem vorkommt, sind Tintenfische bzw. Kopffüßer (z.B. Oktopoden, Kalmare und Sepien). Unter ihnen haben Vertreter der übergeordneten Octopodiformes (i.e. Oktopoden und Vampirkalmare) die Lebensräume von den Gezeiten-Pools bis zur Tiefsee in allen Breitengraden besiedelt. Oktopodiforme Arten aus gemäßigten und tropischen Regionen (Wassertemperatur > 10°C) wachsen typischerweise schnell, leben maximal nur etwa zwei Jahre, erreichen sehr früh die Geschlechtsreife und haben ein einzelnes Fortpflanzungsereignis (Semelparität), nach dem die adulten Tiere sterben. Über die Lebensgeschichte von Tiefsee- und Polararten ist wenig bekannt. Tiefsee- und polare incirrate Oktopoden (flossenlose Arten) sind ebenfalls semelpar, aber sie produzieren große Eier und brüten sie in kaltem Wasser, wodurch die Embryogenese erheblich verlängert wird. Die Vampirkalmare der Tiefsee haben mehrere Fortpflanzungszyklen, für deren Abschluss wahrscheinlich mehrere Monate erforderlich sind. Obwohl die Langlebigkeit der meisten Kaltwasser-Oktopoden unbekannt ist, deuten ihre Lebensdaten auf eine beträchtlich längere Lebensdauer als bei anderen Oktopoden hin, die normalerweise ein Jahr lang leben. Ziel dieser Dissertation ist es, die Langlebigkeit von Tiefsee- und Antarktis-Oktopoden anhand von Lebensdaten und der Quantifizierung von Wachstumsinkrementen in den Schnäbeln (chitinösen Mandibeln) der Oktopoden zu untersuchen.

Zusammenfassung

Im ersten Kapitel werden anhand von veröffentlichten Daten von 26 oktopodiformen Arten, die Entwicklung des Embryos, die Größe der Jungtiere, die Größe und das Alter zum Zeitpunkt der Reife mit den Habitatmerkmalen (Wassertemperatur und Tiefe des Vorkommens) korreliert. Für Arten, deren Alter nicht bekannt war, wurde die Zeit bis zur Reife anhand eines Modells für die Embryonalentwicklung bei niedrigen Temperaturen geschätzt. Die Ergebnisse zeigten eine signifikante negative Korrelation zwischen dem Reifealter und der durchschnittlichen Wassertemperatur. Die Verbreitungstiefe der Tiere korrelierte positiv mit der Zeit bis zur Reife. Die Lebenserwartung gemäßigter und tropischer Arten beträgt in der Regel weniger als 1 Jahr, während antarktische und Tiefsee-Kraken nach 3 bis 5 Jahren reifen. Obwohl die Ergebnisse noch weiterer direkter Validierung bedürfen, stimmen sie mit Lebensdauerschätzungen anderer wirbelloser Gruppen überein, die in der Polar- und Tiefsee leben.

Das zweite Kapitel testet die Hypothese, dass die in der Nähe der Antarktischen Halbinsel lebenden incirraten benthischen Oktopoden (Wassertemperatur -2 bis 2°C) eine längere Lebensdauer haben als ihre gemäßigten und tropischen Verwandten. Das Alter wurde an Exemplaren der Familie Megaleledonidae (*Pareledone* spp., *Adelieledone* spp. und *Megaleledone setebos*) und in *Muusoctopus rigbyae* untersucht, wobei die in ihren Schnäbeln abgelagerten Wachstumszuwächse quantifiziert wurden. Es wurde beobachtet, dass Exemplare in fortgeschrittenen Reifegraden aller Arten häufig mehr als 400 Inkremente in den Schnäbeln aufwiesen. Die Periodizität der Ablagerung für diese Arten ist unbekannt. Die Schnäbel der Weibchen von *Pareledone charcoti*, die 12 Monate lang in Aquarien gehältert wurden, wiesen jedoch 219 bis 364 Wachstumsinkremente auf, das darauf hinweist, dass die schrittweise Ablagerung bei dieser Art länger als einen Tag dauert. Altersschätzungen werden diskutiert und mit anderen Tintenfischarten verglichen, bei denen die Periodizität der Schnabelwachstumsinkremente als täglich validiert wurde.

Das dritte Kapitel verwendet auch die Quantifizierung von Wachstumsinkrementen in den Schnäbeln (Mandibeln), um festzustellen, ob der pelagische Tiefsee-Krake *Japetella diaphana* und der Vampirkalmar *Vampyroteuthis infernalis* im Vergleich zu benthischen Verwandten eine längere Lebensdauer haben. Die maximale Anzahl von Wachstumsinkrementen in den Schnäbeln von Exemplaren von *J. diaphana* und *V. infernalis* betrug 207 bzw. 375. Wenn eine tägliche

Ablagerung der Wachstumsinkremente angenommen wird, stimmen die resultierenden Lebensspannen nicht mit der passiven Lebensweise der beiden Arten überein. Die bemerkenswert niedrigen Stoffwechselraten und die Reproduktionsbiologie dieser Tintenfische lassen eher vermuten, dass die Wachstumszuwächse in den Schnäbeln mehr als einen Tag repräsentieren.

General Introduction

The deep sea, typically defined as the oceans' volume below 200 m, is by far the largest environment on the planet. This vast realm is inhabited by the Earth's most diverse animal communities, most of which remain to be discovered (Robison 2009, Danovaro *et al.* 2014). With human activities such as fishing and extraction of seafloor resources expanding into deeper parts of the ocean over the years, it is becoming increasingly important to establish environmental "baselines", which include not only the documentation of diversity in previously unexplored regions but also the collection of information on life history traits such as age at maturity, growth and longevity (Jones *et al.* 2017). Insight into these traits allow a better understanding of how organisms living under deep-sea conditions (i.e. high pressure, low temperature, low food availability, environmental stability with low disturbance) could respond to anthropogenic stressors, and how their responses may subsequently affect the functioning of deep-sea ecosystems (Smith *et al.* 2008, McClain *et al.* 2012).

The life history traits of cold-water marine ectotherms, such as those from species inhabiting deep seas and polar regions (≤ 5°C), include low fecundity and high longevity (Young 2003, Pörtner *et al.* 2007). Some cold-water taxa can achieve remarkable lifespans, as long as the 400 years for Greenland sharks *Somniosus microcephalus* (Nielsen *et al.* 2016), from 312 to 523 years for the deep-sea oyster *Neopycnodonte zibrowii* (Wisshak *et al.* 2009) and 507 years for the ocean quahog *Arctica islandica* (Butler *et al.* 2013). Long-lived ectotherm species typically grow slow and start to reproduce late in life, which gives them less population resilience when subjected to disturbance (e.g. bottom trawl fisheries), compared to warm water relatives. Examples include the deep-sea fish orange roughy (*Hoplostethus atlanticus,* Collett 1889) and the North Pacific rockfishes (*Sebastes* spp.), which reach maturity after 20 yr of age and can live for over 125 yr (Cailliet *et al.* 2001, Norse *et al.* 2012). These species are commercially valuable and their populations, when subjected to high fishing pressure, have suffered from rapid declines due to their slow pace of life (Clark *et al.* 2016). The environmental and ecological constraints (e.g., temperature, depth, light, food availability, predation pressure) driving these life history trends remain not fully comprehended. One potential impact of overfishing on long-lived finfish species may be the increase in populations of

opportunistic, short-lived invertebrate competitors (Caddy & Rodhouse 1998). Examples of such opportunistic marine invertebrates are the cephalopod mollusks (Class Cephalopoda, i.e. squids, octopuses and cuttlefishes).

The metabolic rates of marine ectotherms show a strong decline with increasing depth of occurrence even after correcting for temperature and size effects (Childress 1995), and metabolic activity in some deep-sea organisms can be hundredfold lower than in shallow-water relatives (Seibel & Drazen 2007). This trend is more pronounced in pelagic organisms inhabiting meso- and bathypelagic depths (200-3000 m) than in benthic organisms (Seibel & Childress 2000). The strong decline in metabolic rates observed in pelagic organisms that rely on vision for orientation (e.g., fishes and cephalopods), lead to the ‘‘visual-interaction hypothesis’’ (Childress *et al.* 1990). This hypothesis suggests that animals that are dependent on vision and light for predator-prey interactions have higher energetic demands and hence higher metabolic rates (Childress 1995, Seibel & Drazen 2007). In the bathypelagic zone where light is absent, predator-prey detection abilities are reduced and so are the locomotory capacities for chasing prey or escape from predators, resulting in body types that are more gelatinous than muscular and which have lower energetic demands (Seibel & Drazen 2007). The environmental stability and reduced predation pressure associated to the decrease in temperatures and food availability (i.e. caloric restriction), may have favoured life-history traits which include late maturity and long lifespans (Stearns 1992). Overall more research on comparison between life history strategies between shallow-living and deep-sea ecological important organisms is necessary. A Class of organisms that commonly inhabits the cold waters of polar and deep seas, but in which most of the research on life history traits has been restricted to shallow-water forms is the Cephalopoda (e.g., octopods, squids, cuttlefishes and nautiluses).

Cephalopods inhabit all oceans at every latitude and they occur from tide pools to abyssal depths (Hoving *et al.* 2014, Jereb *et al.* 2016, Purser *et al.* 2016). Most squids and cirrate (i.e. finned) octopods adopted a pelagic lifestyle, while incirrate (i.e. unfinned) octopods and sepioids are typically associated with the bottom (Boyle & Rodhouse 2005). The deepest living cephalopod caught on camera is the Casper octopus, which was encountered at >4200 m (Purser *et al.* 2016). Cephalopoda are particularly abundant in the deeper parts of the oceans, where they show the greatest diversity at the family level and total biomass of cephalopods

(Hoving *et al.* 2014). The group is an important link in the food chain, preying on zooplankton, micronekton and benthic invertebrates (e.g., crustaceans, echinoderms, fish, other mollusks) (Villanueva *et al.* 2017), and are prey for high predators like fishes, cetaceans and seabirds (Clarke 1996, Croxall & Prince 1996, Smale 1996).

Historically, life cycle studies on cephalopods have focused on the most common, near-shore forms of squids, octopods and cuttlefishes, which have consistently revealed short lifespans, rarely exceeding ~1-2 years (Arkhipkin 2004, Jackson 2004, Semmens *et al.* 2004). The majority of species grow fast and have a single reproductive cycle dying soon after their last reproductive event (semelparity), exceptions being the shelled nautiluses, the Antarctic giant warty squid *Kondakovia longimana* and the vampire squid *Vampyroteuthis infernalis* (Rocha *et al.* 2001, Laptikhovsky *et al.* 2013, Hoving *et al.* 2015). Among the oceanic squids from the suborder Oegopsida, lifespans tend to increase with latitude and adult size, varying from 3-5 months in small-sized tropical species to 3 years in large species inhabiting temperate and subpolar–polar latitudes (Arkhipkin 2004). Age and size at maturity is coupled with environmental abiotic and biotic factors and can vary within species (Pecl & Jackson 2008, Hoving *et al.* 2013). The lifespan of the ommastrephid squid *Todarodes pacificus* (Steenstrup, 1880), can be reduced by up to 50% under higher than normal temperature regimes (Takahara *et al.* 2017).

Some cephalopod species have very flexible life history strategies; they can adapt quickly to environment and ecosystem changes, *e.g*. by reaching full maturity at different sizes and ages depending on the productivity and physical characteristics of the environment (Hoving *et al.* 2013). A few studies suggested that overexploitation of finfish stocks may have resulted in a niche availability that favored cephalopod populations, leading to regional increases in their biomass over the last 60 years (Caddy *et al.* 1998, Zeidberg & Robison 2007, Hunsicker *et al.* 2010, Doubleday *et al.* 2016). This was also observed in the Southern Ocean by Vecchione *et al*. (2009), who argued that overfishing of finfish target species in late 1970´s around Elephant Island (South Shetland Islands off the Antarctic Peninsula), could be responsible for the high abundance of octopods collected in that area by research cruises from late 1980´s to recent years. If these assumptions are correct, changes in such sensitive ecosystems could last for many years, especially considering that ecological processes in polar regions tend to be comparatively slow (Peck 2016).

Over the last few years, there has been increasing direct and indirect evidence which suggest high longevities for deep-sea cephalopods compared to their shallow water relatives. The lifespan of the Southern Ocean colossal squid *Mesonychoteuthis hamiltoni* is unknown, but it lives in temperatures of 1.7°C and has low metabolic and energetic requirements, suggesting that large specimens (max. weight > 400 kg) may live for several years (Rosa & Seibel 2017). The deep-sea squids *Octopoteuthis deletron* and *Galiteuthis phyllura* have slow growth rates and reach maturity after 3 years (Hoving & Robison 2017). The reproductive strategy may also extend the total lifespan of a cephalopod, and some deep-sea squid species (e.g. *Bathyteuthis berryi*, *Gonatus onyx*) hold on to their eggs after spawning and brood them in the cold bathypelagic zone (~2-4°C), where embryonic development can take 9-12 months (Seibel *et al.* 2000, Bush *et al.* 2012). Considering that the female needs to care for the eggs until the embryos hatch, this post-spawning egg care period seems to exceed the total lifespan of many neritic squid species (Boletzky 1994, Seibel *et al.* 2000). Extended lifespans may also occur in cephalopods adapted to live permanently over depths where oxygen is near depleted, exhibiting restrained mobility and low metabolic rates in a very cold environment. Pelagic deep-sea octopods (family Bolitaenidae) also brood their eggs in bathypelagic depths > 1000 m (Young 1972, 1978). The females of the deep-sea vampire squid *V*. *infernalis* show evidence of multiple reproductive cycles during their lifetime, such strategy associated to environmental and biological constraints (i.e. cold water, low oxygen, caloric restriction and low metabolic rates) indicate that lifespan in this species may take several years (Hoving *et al.* 2015).

Another remarkable example of increased longevity and extreme parental care was the observation of the brooding time of the deep-sea octopus *Graneledone boreopacifica*, on a rocky outcrop at 1397 m deep in the Monterey Submarine Canyon (California, USA) (Robison *et al.* 2014). In this peculiar case, scientists used remotely operated vehicles (ROVs) to document in situ during 53 months the brooding of one female octopus and the embryonic development of the eggs (Robison *et al.* 2014). To date, *Graneledone* holds the record for the longest embryonic development period in the animal kingdom. This observation confirmed earlier suggestions that female octopuses of *Graneledone* spp. brood their benthic eggs for long periods throughout development (Voight 2000, Voight & Grehan 2000). If the embryonic phase is one third of the total life cycle, then octopuses of this genus

might be the record holders in longevity among coleoid cephalopods (Boletzky 1987, Robison *et al.* 2014). Moreover, recent observations of different deep-sea octopod species brooding larger eggs than those of *G. boreopacifica* in deeper and colder waters, suggest that embryonic development could even last longer for other species (Purser *et al.* 2016, Hartwell *et al.* 2018).

Antarctic benthic octopods also produce large eggs (15-40 mm) which may require several years of embryonic development in temperatures of -2 to 2°C (Boletzky 1994, Allcock *et al.* 2003, Allcock 2005). Most of the octopod species endemic to the Southern Ocean belong to the family Megaleledonidae, which contains *Graneledone* spp. and other deep-sea genera inhabiting other ocean regions (Strugnell *et al.* 2008, 2014). The Antarctic *Pareledone charcoti* has the lowest growth rates ever measured for a benthic octopod species, with a captive specimen growing 10 g in 18 months (Daly & Peck 2000). Mature specimens of the species *Megaleledone setebos* can weigh as much as 25 kg and lay eggs that are ~ 40 mm (Allcock *et al.* 2003, Piatkowski *et al.* 2003). The life history traits of Antarctic octopods indicate that their lifespans may be considerably longer than shallow-water species from temperate and tropical regions. Extended longevity compared to temperate and tropical organisms has been observed in many Antarctic invertebrates including echinoderms, asteroids, bivalve and gastropod molluscs (Peck 2016).

Overall, longevity in deep-sea and polar cephalopods is still very poorly known. However, size-at-age is a crucial parameter for stock assessment models, for the understanding of population turn-over rates and hence the production of ecosystems. Such information is valuable for evaluate direct environmental impacts (e.g. fishing and mining) or indirectly linked to anthropogenic actions (e.g. climate change), and the implementation of conservation strategies for deep-sea species and ecosystems. All over the world, exploitation of natural resources is expanding which makes studies on the life history of deep-sea species even more relevant (Morato *et al.* 2006, Hein *et al.* 2010, Thurber *et al.* 2014).

Estimation of age and growth in cephalopods can be done via the quantification of growth increments in specific hard body parts. Examples of these structures are the chitinous beaks, eye lenses, statoliths (aragonite concretions inside the statocysts, the organs of balance), the gladius (chitinous internal ‘pen’) and the cuttlebone (internal shell used for buoyancy) (Boyle & Rodhouse 2005). The age determination of wild squids and cuttlefish has been done via the quantification of

growth increments in sectioned statoliths and/or gladius. Although for decapodiforms such efforts have presented good results, the same proved not to be true for octopods (Lombarte *et al.* 2006). In octopuses, statoliths are soft, chalk-like and appear to lack growth increments (Clarke 1978, Robinson & Hartwick 1986). The gladius and cuttlebone are absent in octopuses. Instead, octopods have a pair of vestigial internal shells named stylets, which are two fine elongated rod-like cartilaginous structures present inside the mantle muscle at the base of the gills. These structures have been used as tools for age determination in coastal octopuses (Arkhipkin *et al.* 2018). Increment validation and quantification showed that growth increments in stylets and beaks are deposited on a daily basis (Doubleday *et al.* 2006, Hermosilla *et al.* 2010, Rodríguez-domínguez *et al.* 2013, Perales-Raya *et al.* 2014). For deep-sea octopuses the periodicity of growth increments deposition is still unknown (Barratt & Allcock 2010). In most Antarctic octopod species, the stylets are very fragile and not suitable for age determination, and in the deep-sea pelagic octopod families Amphitrethidae, Bolitaenidae and Vitreledonellidae stylets are absent (Barratt & Allcock 2010, Norman & Finn 2016). However, all extant cephalopods have chitinous beaks that continuously deposit new layers of chitinous material as the animals grow, and which are preserved in the form of growth increments. These structures may serve as “life recorders”, exhibiting different deposition patterns that can be related to seasonal events (Canali *et al.* 2011), and provide ecological information even after retrieved from predator stomachs (Cherel & Hobson 2005).

Thesis Outline

In order to better understand the life cycles of cold water octopods (< 5° C), and to assess how vulnerable or flexible these cephalopods are, this thesis aims to investigate size-at-age, growth rates and longevity in Antarctic and deep-sea octopodiform species. Potential age and lifespans were estimated using life history traits and the quantification of growth increments in the octopods’ beaks. As study material, we used a unique collection of benthic Antarctic octopods, the pelagic octopus species *Japetella diaphana* (Hoyle 1885) and the vampire squid (*V. infernalis* Chun 1903). In this dissertation three major questions have been addressed:

1. **Are octopod lifespans inversely correlated with habitat temperature**?

The first chapter of the thesis aims to investigate how octopod life-history traits including information on hatchling and maturity sizes, embryonic development times and time to reach maturity are correlated with habitat characteristics (temperature and depth of occurrence). The data used in the analyses came from published literature from 25 benthic and pelagic octopod species and the vampire squid. The hypothesis tested is that octopod life-history characteristics are correlated with habitat temperatures, and cold water species may exhibit extended lifespans.

2. **Do Antarctic octopod species have longer lifespans than tropical and temperate counterparts**?

In the second chapter the main objective was to test if benthic octopods living in the vicinity of the Antarctic Peninsula have a slower pace of life compared to their temperate and tropical relatives. Specimens from the families Megaleledonidae (*Adelieledone* spp., *Pareledone* spp. and *Megaleledone setebos*) and Enteroctopodidae (*Muusoctopus rigbyae*; Vecchione, Allcock, Piatkowski & Strugnell 2009) were collected on the shelf and slope regions off the Antarctic Peninsula during a cruise in 2012. Examined specimens included early juveniles to animals in advanced maturity. The methodology used to investigate the animals' age was the quantification of growth increments deposited in their chitinous beaks. Growth increments were quantified in 266 beaks of four species (*Pareledone aequipapillae*, *P*. *charcoti*, *Megaleledone setebos* and *Muusoctopus rigbyae*). Eleven females of the species *Pareledone charcoti* (Joubin, 1905) were captured during the same cruise and kept under laboratory conditions for ~12 months. The beaks of these individuals were examined to compare the number of growth increments with the elapsed captivity time.

3. **Do deep-sea pelagic octopodiforms have a slow pace of life compared to benthic relatives**?

The third chapter aimed to assess if the deep-sea pelagic octopodiforms *Japetella diaphana* and *Vampyroteuthis infernalis* have longer lifespans compared to benthic relatives. These two species are known to inhabit the dark and cold meso- and bathypelagic zones in the world's tropical and temperate latitudes, feeding upon a non-typical cephalopod diet that includes detritus, and maintaining a lifestyle with low energy expenditure with reduced locomotion and low metabolic rates. The

characteristics of their life strategies suggest a slow pace of life, which may result in lifespans exceeding the typical ~2 years observed in most coleoid cephalopods. Age was estimated using methods similar to those applied in the second chapter. Reproductive aspects of *J. diaphana* were investigated examining animals in all maturity stages, from juvenile to post-spawning (brooding). Although the periodicity of beak growth-increments is unknown for the two species, this might be the first attempt to use growth increments in hard body parts to indirectly estimate age in pelagic octopodiforms.

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Chapter 1

Impact of environmental temperature on the lifespan of octopods

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1.1 Abstract

Recent studies on the life history of cephalopods have challenged the paradigm that all coleoid cephalopods have a single reproductive cycle and a short lifespan. Although lifespan has been investigated in several octopod species, few studies have considered their life-history traits in relation to environmental conditions via a comparative approach. We tested the hypothesis that octopod lifespan is correlated with habitat characteristics. For that purpose, life history and environmental data of 25 incirrate octopod species and the vampire squid *Vampyroteuthis infernalis* were compiled from the literature. Regression analysis showed that the relationship between age at maturity and average habitat temperature was best described by a negative power function (r^2 = 0.86). The depth ranges of occurrence (minimum-midpoint-maximum) were positively correlated with time to reach maturity, with maximum depth showing the best fit (r^2 = 0.47). Using literature data and our analyses, we estimated that octopods living in polar and deep seas mature after 3 to 5 yr. The reviewed and estimated instantaneous relative growth rates ranged from 0.1% body weight (BW) d^{-1} in the Antarctic species *Pareledone charcoti* to nearly 6% BW d^{-1} in the temperate species *Macroctopus maorum*. Our analyses suggest that low water temperatures (< 5°C) result in an extended ontogenetic development, probably as a result of reduced metabolic rates and constraints on protein synthesis, which increases the lifespan of octopods living in cold environments.

KEY WORDS: Cephalopoda · Incirrate octopods · Life-history traits · Temperature · Lifespan · Deep sea · Polar regions

1.2 Introduction

A general trend in marine organisms is that the pace of life is reduced for species living in cold-water environments. Metabolic and growth rates are influenced by temperature, with organisms developing, growing, maturing and ageing faster at higher temperatures (Atkinson 1994, 1996, Angilletta *et al*. 2004). Marine poikilotherms such as fishes and invertebrates that live in polar and deep seas exhibit increased longevities when compared to their relatives living in temperate or warm waters (Cailliet *et al*. 2001, Young 2003). Research on various deep-sea taxa has revealed extreme longevities, including 11 000 years (yr) for the deep-sea sponge *Monorhaphis chuni* (Jochum *et al*. 2012), 4 000 yr for proteinaceous corals (Roark *et al*. 2009), between 312 and 523 yr for the deep-sea oyster *Neopycnodonte zibrowii* (Wisshak *et al*. 2009), 507 yr for the ocean quahog *Arctica islandica* (Butler *et al*. 2013), 400 yr for Greenland sharks *Somniosus microcephalus* (Nielsen *et al*. 2016) and 200 yr for the rockfish *Sebastes aleutianus* (Cailliet *et al*. 2001). For most deep-sea taxa, information on longevity and other life-history characteristics is lacking, and hence it remains unknown how these parameters are related to habitat temperatures.

Cephalopods (e.g. squids and octopods) occur at all latitudes and inhabit all marine habitats, from tide pools to abyssal depths (Boyle & Rodhouse 2005). As adults, most cephalopods are active carnivores that hunt for living prey, and typically they have one reproductive cycle (semelparity). Cephalopods are very abundant in the deep ocean, where large populations exist and where they show a great diversity at the family level (Rosa *et al*. 2008, Hoving *et al*. 2014). They are important in food webs as prey (e.g. marine mammals, top predatory fish, seabirds) and predators (e.g. on crustaceans, fishes and other molluscs) (Clarke 1996, Piatkowski *et al*. 2001, Villanueva *et al*. 2017). A recent study suggests that the overexploitation of finfish stocks may have favoured cephalopod populations, leading to regional increases in their biomass over the last 60 yr (Doubleday *et al*. 2016). Despite the pivotal role of cephalopods in the world oceans, knowledge on life histories is lacking for the majority of species that inhabit the deep sea.

Longevity studies on neritic cephalopods have consistently revealed short lifespans, rarely exceeding 3 yr (Arkhipkin 2004, Jackson 2004). The short lifespan is combined with a semelparous reproductive strategy. Semelparity in cephalopods means that all gametes are released during a single reproductive cycle, without a

gonadal resting phase (Rocha *et al*. 2001). With the exception of the Antarctic giant warty squid *Kondakovia longimana* (Laptikhovsky *et al*. 2013) and the vampire squid *Vampyroteuthis infernalis* (Hoving *et al*. 2015), all extant coleoid cephalopods that have been studied to date are semelparous. Within the borders of semelparity, the spawning strategies range from terminal synchronous spawning to the intermittent spawning of egg batches, with specific differences between cephalopods in fecundity, egg size, batch size, time and presence or absence of somatic growth between spawning events, mortality in ontogenetic phases and embryonic development time (Rocha *et al*. 2001, Hoving 2008).

Embryonic development in cephalopods ranges from a few days in coastal and tropical warm-water species to several months or even years in deep-sea and polar species (Boletzky 1994, Boyle & Rodhouse 2005, Robison *et al*. 2014). Intraspecific lifespan plasticity has been demonstrated for some species with wide distribution ranges, e.g. ommastrephids. In these species, the life cycle duration increases with latitude and adult size, varying from 5 months (mo) in small-sized tropical forms to more than 1 yr in large animals that live in temperate and subpolar–polar latitudes (Arkhipkin 2004). Furthermore, cephalopods can adapt rapidly to local environmental and ecosystem changes by reaching full maturity at different sizes and ages depending on the productivity of the environment (Pecl & Jackson 2008, Hoving *et al*. 2013, Schwarz & Perez 2013, Arkhipkin *et al*. 2015). Overall, there is evidence that the pace of life in cephalopod species is coupled strongly with the environment (Rodhouse *et al*. 2014, Hoving & Robison 2017).

Lifespan is also related to the reproductive strategy of cephalopods. Some deep-sea squids (e.g. *Gonatus onyx*, *Bathyteuthis berryi*) retain their eggs after spawning and brood in the bathypelagic zone, out of reach of bottom- and surface-dwelling predators (Seibel *et al*. 2005, Bush *et al*. 2012). In cold-water environments (<5°C), embryonic development is slow and may last for several months or even years (Boletzky 1994, Seibel *et al*. 2000). Using deep-sea observations from remotely operated vehicles, an extremely long brooding time was discovered for the deep-sea octopus *Graneledone boreopacifica* in the Monterey Submarine Canyon off California, USA. One brooding female was monitored from the first weeks when spawning of the eggs took place until the last weeks when the eggs hatched. The embryonic development lasted 53 mo, the longest brooding time ever recorded (Robison *et al*. 2014). Following the assumption that brooding time makes up 30% of

the total life cycle (Boletzky 1987), this cold water octopus is expected to live >10 yr, a result that challenges the paradigm that cephalopods are short-lived animals. The few studies that investigated the lifespan of cold-water cephalopods including deep-sea species show increased longevities and reduced growth compared to warmer-water species (Arkhipkin 2004, Robison *et al*. 2014, Hoving & Robison 2017), but comparative studies on a wider range of species are still rare.

One of the first reviews on cephalopod growth and lifespan in relation to environmental factors was performed by Wood & O'Dor (2000). The authors investigated the effects of temperature and phylogeny on size at maturity and longevity, using literature data of 18 species from 5 cephalopod orders. The authors concluded that low temperatures delay the time to reach maturity and that larger cephalopods have longer life spans. However, the study was biased towards coastal cephalopods and included only 2 cold-water species, the North Atlantic octopus *Bathypolypus arcticus* and the giant Pacific octopus *Enteroctopus dofleini*. Seibel (2007) measured metabolic rates of pelagic cephalopod species from 8 families all inhabiting different environments. The study showed that the active and muscular coastal and epipelagic squid families Loliginidae and Ommastrephidae have the highest metabolic rates of all invertebrates, while the less active and gelatinous cold-water meso- and bathypelagic octopods (Bolitaenidae and vampire squids) had metabolic rates up to 200-fold lower than their shallow-living relatives. Since the high metabolic rates of ommastrephids are associated with a short life cycle, it may mean that cephalopods with low metabolic rates are longer lived, but data to support this notion are scarce.

In cephalopods, egg sizes and numbers, embryonic development time and the duration of the planktonic larval phase are dependent on habitat temperatures (Boletzky 1994, Voight & Drazen 2004, Laptikhovsky 2006, Villanueva & Norman 2008), which together can be used to estimate lifespan (Robison *et al*. 2014). Incirrate octopods occur in all marine habitats and they provide post-spawning egg care, retaining the eggs during the entire development period (Boletzky 1992, Jereb *et al*. 2014). While incirrate octopods are a very suitable model group to investigate how environmental temperature may impact longevity, this has never been done in a comparative approach. We performed regression analysis of life-history data of incirrate octopods and the vampire squid to test the hypothesis that octopod lifespans are inversely correlated with habitat temperature, i.e. octopods living in cold-water

habitats of the polar and deep seas have increased longevity compared to their shallow-water relatives.

1.3 Materials and Methods

1.3.1 Data extraction from the literature

An extensive literature review resulted in life-history data for 25 species of incirrate octopods (Cephalopoda: Octopoda - Incirrata) as well as the vampire squid (Table 1). We used benthic and pelagic species with a distribution ranging from tropical waters to polar seas and from shallow coastal (0–15 m) to bathyal depths (3000 m). For 2 species (*Pareledone charcoti* and *Bathypolypus arcticus*), we considered anecdotal data obtained from long-term husbandry of octopods under controlled laboratory conditions (Wood 2000, Daly & Peck 2000).

When a life-history trait (e.g. hatchling size) was available from more than 1 source for the same species (e.g. *Octopus vulgaris*), an average value for the species was calculated. When averages of biological and life-history traits (Table 1) were not explicitly listed, we calculated them by taking the midpoint between published minimum and maximum parameter values. For species where literature about life-history traits is scarce, we pooled information for the genus (e.g. *Graneledone boreopacifica* and *G*. *macrotyla*). We used the average value representative of the species or genus and followed the FAO taxonomic criteria for the latest taxonomic status of taxa (Jereb *et al*. 2014, Villanueva *et al*. 2016). All data values, with the exception of *P*. *charcoti*, refer to females.

1.3.2 Calculations

Life-history traits were quantified following the methodology applied by Wood & O'Dor (2000). Size at maturity was defined as the weight at full maturity (g), and age at maturity was noted in days (d). Instantaneous relative growth rate *G* (% body weight d^{-1}) was calculated from the formula:

$$G = \frac{ln(W_2) - ln(W_1)}{(t_2 - t_1)} . 100 \quad (1)$$

where W_2 is the weight at maturity, W_1 is the weight at hatching and (t_2 - t_1) is the time interval between hatching and maturity (Forsythe & Van Heukelem 1987).

Table 1. Life history data and references for the octopod species analysed in the present study. Superscript numbers refer to literature from where the values were obtained (for references, see the Supplement S1 in this thesis' Appendix; or https://www.int-res.com/articles/suppl/m605p151_supp.pdf). **Abb**: Abbreviation of the species names; **Depth range**: minimum and maximum depth for the species; **Max ML**: maximum mantle length; **Emb.dev**.: duration of the embryonic development; **~Emb.temp**.: average temperature of egg incubation; **Hatch.size**: size at hatching (ML); **Hatch.weight**: weight at hatching; **Mat.wt**: weight at maturity; **Mat.ML**: ML at maturity; **Age mat**.: age at maturity; **Average lifetime temp**: temperature for the species; **Lifespan**: estimated in days; ***G***: instantaneous relative growth rate; **Method**: method of age estimation, where L: laboratory, F: field experiments, B: beaks, St: stylets, Lp: lipofuscin concentration, Eye: eye lenses, *In situ* observations and Rep: reproductive behavior and time for egg incubation. Values in ***bold italics*** were estimated (see 'Materials and methods'). Genus (***av.***): average values obtained from different reference sources (see 'Data extraction from the literature' in 'Materials and methods')

Species	Abb	Region	Depth Range (m)	Mantle length (max)	Egg Size (mm)	Emb. dev. (days)	~Emb. Temp. (°C)	Hatch Size (mm)	Hatch. weight (g)	Mat W (g)	Mat ML (mm)	Age Mat. (d)	Average Lifetime temp(°C)	Life-span (d)	*G*	Method
Octopodidae																
Bathypolypus arcticus	*Ba*	North Atlantic	20-1200[1,4]	70[1]	18.0[2,4]	420[2]	7[2]	8.9[2]	0.214[2]	70[1]	57[3]	1400[1]	4.0[1]	***1820***	0.41[1]	L, F
B. sponsalis	*Bs*	Northeast Atlantic and Mediterranean	930-1250[4]	100[4]	15.0[5]	***126***	12[7]	7.7[8]	***0.151***	***150***	58[6]	330[6]	12.0[7]	***456***	2.09	St
Eledone cirrhosa	*Ec*	Mediterranean and North Atlantic	30-500[4,10]	250[4]	7.6[9]	110[9]	16[9]	4.5[9]	***0.047***	950[10]	174[10]	418[10]	16.0[9]	517[10]	2.37	L,St
Eledone massyae	*Ema*	Brazil - Argentina	0-300[4]	90[11]	12.0[11]	***96***	14[11]	10.5[11]	***0.296***	108[11]	90[11]	***540***	14.0[11]	***720***	1.09	Rep.
Enteroctopus dofleini	*Ed*	North Pacific (Alaska- Japan)	0-1500[4]	600[4]	8.0[4]	180[4]	10.8[8]	5.4[8]	0.025[1]	16300[1]	600[4]	1019[12]	10.7[1]	***1136***	1.31	L,F
E. megalocyathus	*Emg*	Chile to Argentina	15-170[13]	190[4]	10.7[14]	176[15]	12[13]	8.4[14]	0.111[14]	1613[13]	158[13]	558[16]	12.5[15]	***734***	1.72	L, Eye
Graneleone boreopacifica; G. macrotyla	*Gr*	South hemisphe Northern Pacific	400-3000[4]	145[17]	15[17-20]	1590[18]	3.3[18]	28[17]	2.5[17]	811[19-20]	141[18,20]	***3180***	3.3[18]	***4770***	0.18	In situ ,Rep.
Macroctopus maorum	*Mo*	Pacific Australia New Zealand	0-50[21]	300[4]	7.0[21]	60[21]	18[21]	3.3[21]	0.009[22]	6000[22]	-	224[22]	18.0[21]	365[22]	5.97	St
Pteroctopus tetracirrhus	*Pt*	Mediterranean North Atlantic	200-500[23]	140[23]	10[24-25]	90[24]	12[23]	8.3[26]	***0.178***	476[25]	122[23]	365[***23***]	12.0[23]	***455***	2.16	Rep.
Octopus bimaculoides	*Ob*	NE Pacific California	0-20[4]	120[27]	18[4]	46[27]	23[8]	6.5[8]	0.070[27]	597[27]	120[27]	370[27]	23.0[27]	***416***	2.45	L, F
O. cyanea	*Oc*	Tropical West Pacific East Africa to Hawaii	0-25[4]	160[4]	2.8[26,28]	23[26,28]	26[26,28]	2.1[29]	***0.009***	2883[28,30,31]	156[30,31]	250[28-31]	25.6[28-31]	411	5.09	L,F,St

Table 1. (continued)

Species	Abb	Region	Depth Range (m)	Mantle length (max)	Egg Size (mm)	Emb. dev. (days)	~Emb. Temp. (°C)	Hatch Size (mm)	Hatch. weight (g)	Mat W (g)	Mat ML (mm)	Age Mat. (d)	Average Lifetime temp(°C)	Life-span (d)	*G*	Method
O. joubini	*Oj*	Gulf of Mexico Caribbean Sea	0-15[32]	45[4]	3.5[32]	35[32]	25[32]	2.5[32]	0.003[32]	30[32]	45[32]	182[32]	25.0[32]	***217***	5.08	L, F
O. maya	*Om*	Gulf of Mexico	0-50[1,4]	125[4,33]	17.0[4]	50[33]	25[33]	7.0[33]	0.120[1,33]	1799[1,33-34]	126[33]	234[33]	25.0[33]	***284***	4.12	L,F
O. pallidus	*Op*	Australia	0-200[35-40]	150[4,35]	11.4[37-38]	136[37,39]	13.5[35-40]	6.5[8]	0.276[37]	583[35-40]	-	246[35-40]	15.8[35-37]	***437***	3.12	St,Lp
O. tetricus	*Ot*	Australia	0-50[41,42]	182[42]	2.6[4,43]	50[41,43]	16[41,43]	2.0[41,43]	***0.008***	2027[41,42]	155[41,42]	293[41,42]	19.0[41-43]	***381***	4.25	L,St
O. vulgaris	*Ov*	Worldwide tropical to temperate	0-250[1,4]	200[44-48]	2.0[4,8]	30[44,46]	20[44-48]	2.0[44,45]	0.003[44,45]	2535[44-48]	188[44-48]	382[44-48]	20.0[44-48]	***411***	3.54	L,B,St
Robsonella fontanianus	*Rf*	South America (Peru to Chile)	0-200[4]	70[4,49]	5.2[50]	74[51]	12[51]	3.0[50]	***0.019***	82[50]	62[50]	280[51]	12.0[51]	424[51]	2.98	L
Megaleledonidae																
Adelieledone polymorpha	*Ap*	Antarctic - South Shetland Islands	15-365[52]	105[53]	16.5[53]	***1590***	1.0[54]	***9.9***	***0.261***	143[52]	73[52]	***3180***	1.0[54]	***4770***	0.20	Rep.
Pareledone charcoti	*Pc*	Antarctic - South Shetland Islands	48-392[55]	65[55]	14.0[55]	***1590***	1.0[54,56]	***8.7***	***0.197***	74[56]	-	***3996***	1.0[54]	***3996***	0.15	L
P. turqueti	*Ptu*	Antarctic to South Georgia	25-1000[57]	96[53]	19.0[53]	***1590***	1.0[54]	***11.1***	***0.332***	236[53]	86[53]	***3180***	1.0[54]	***4770***	0.21	Rep.
P. aequipapillae	*Pa*	Antarctic Peninsula	110-465[55]	70[55]	14.0[55]	***1590***	1.0[54]	***8.7***	***0.197***	90[55]	56[55]	***3180***	1.0[54]	***4770***	0.19	Rep.
Megaleledone setebos	*Ms*	Circum-Antarctic	32-850[4]	280[4]	41.5[58]	***1590***	1.0	***20.5***	***1.268***	7800[6,59]	190[6,59]	1077[6]	1.0	***2667***	0.81	St
Vampyromorpha																
Vampyroteuthis infernalis	*Vi*	Tropical and subtropical waters	600-1500[60]	210[61]	4.5[61]	***731***	4.5[61]	8.0[62]	***0.164***	448.0[60]	102[60]	***1462***	4.5[61]	***2193***	0.72	Rep.
Bolitaenidae																
Japetella diaphana	*Jd*	Tropical and subtropical waters	200-1000[4]	85[4]	3.0[63]	***731***	4.5[63]	2.5[63]	***0.013***	-	55[64]	***1462***	4.5[63]	***2193***	-	Rep.
Bolitaena pygmaea	*Bp*		100-1400[4]	60[4]	2.0[63]	***731***	4.5[63]	2.0[65]	***0.008***	-	45[65,66]	***1462***	4.5[63]	***2193***	-	Rep.

1.3.3 Embryonic development

Embryonic development times were taken as the average values obtained from experiments (Wood 2000, Iglesias *et al*. 2014). For species with no direct information on embryonic development, the values were estimated following the equation:

$$D = 10794T^{-1.79} \quad (2)$$

which predicts the embryonic developing time in days (D), based on the average habitat temperature in °C (T) (Robison *et al*. 2014). The authors compiled this equation using several sources of information including direct observations on embryonic development of *G. boreopacifica*, which lasted 1590 d at 3°C (53 mo).

Unfortunately, there is a limitation when applying this equation to estimate embryonic development times for Antarctic octopods because they typically live in water temperatures ranging from -2° to 2°C (Kuehl 1988). The equation does not processes negative temperature values, and even at a water temperature of 1°C, the predicted time for embryonic development would be about 30 yr, which seems unrealistic. Since the egg dimensions of Antarctic octopods and those of *G. boreopacifica* are similar, we considered embryonic development time for octopod species brooding at water temperatures below 3°C to be 53 mo (sensu Robison *et al*. 2014). These estimates are conservative, since Antarctic octopods brood in an average temperature of 0°C (Kuehl 1988).

The hatchling size in relation to the adult size was also explored as a predictor for lifespan. We calculated the species hatchling size index (SHSI, %) as: (hatchling ML)/(maximum adult ML) × 100 (Villanueva *et al*. 2016), where ML is mantle length.

1.3.4 Age at maturity

Age at maturity was chosen as a lifespan indicator assuming that for the majority of species, growth is reduced or completely halted after spawning. With the exception of the vampire squid, all species included in this study are assumed to have 1 reproductive cycle and die shortly after the last eggs have been released or when the offspring hatches.

Age at size data were obtained from direct observations (e.g. laboratory or culture studies) and from estimates using indirect methods such as the interpretation of the growth increments in eye lenses, beaks and stylets, or the quantification of the

lipofuscin concentration (Table 1). When data from 2 methods were available for the same species (e.g. beaks and stylets) an average value was used to estimate age.

We used the predicted embryonic development times (*D*) to estimate age at maturity for the deep-sea pelagic species *Vampyroteuthis infernalis*, *Bolitaena pygmaea* and *Japetella diaphana*, for the benthic deep-sea genus *Graneledone* (*G*. *boreopacifica* and *G*. *macrotyla*) and for the Antarctic species *Adelieledone polymorpha*, *Pareledone aequipapillae* and *Pareledone turqueti*. The period of brooding in incirrate octopods, which brood large eggs (>10 mm) under low temperatures, may cover up to one third of the female's life cycle (Boletzky 1987). Therefore, we estimated age at maturity as 2 times the predicted embryonic development duration (2×*D*) and the total lifespan as 3 times this period (3×*D*). For example, the vampire squid and bolitaenids were assumed to reach maturity after 4 yr (i.e. 2×*D* = 1462 d), and lifespan was calculated as 3×*D* (*D* = 731 d; lifespan = 3×731 d), which resulted in about 6 yr. These assumptions are based on the best data available, which are very limited and the lifespans proposed here will need to be validated or refuted in future studies.

To estimate lifespan for the Antarctic Charcot's octopus *P. charcoti*, we used the average *G* (% body weight d^{-1}) values obtained from specimens that were collected in Antarctic waters and were kept in captivity for 21 mo (Daly & Peck 2000). In that study, 1 of the male octopods grew 10 g in 18 mo (from 64 to 74 g) and subsequently died after the release of 1 large spermatophore. The growth rate of that specimen was reported as 0.1% body weight d^{-1}. Assuming that *G* was constant since the hatchling phase, the animal was about 11 yr old (i.e. 3996 d). Adult animals typically exhibit reduced growth rates compared to juveniles and this would explain such low values for *G*. Even though this value is a rough estimation, an octopod from the same genus and the same area was kept alive at the Alfred Wegner Institute in Bremerhaven, Germany, for about 8 yr (F. Mark unpubl. data). This lifespan also fits in the longevity expected for other cold-water octopods (Wood 2000, Robison *et al*. 2014). When age estimates were available in the literature, the lifespan was considered as the sum of the period to reach maturity and the embryonic development time.

1.3.5 Hatchling size and weight

For the Antarctic octopods *A. polymorpha*, *Megaleledone setebos* and *Pareledone* spp., data on hatchling sizes (HTs) and hatchling weights (HTw) are not

available. To estimate hatchling sizes in these species, a model was fitted using data available for egg sizes and hatchling size from 20 species used in the present study, including the pelagic deep-sea species *B. pygmaea*, *J. diaphana* and *V. infernalis*. The regression parameters were estimated by the minimization of the least squares error sum. The model that best described the relationship between egg size (*EgS*) and hatchling size (*HTs*) was expressed by a power function: HTs = $1.086EgS^{0.788}$ (r^2 = 0.70, p < 0.001; Fig 1). For *G. boreopacifica*, we used the egg size (15 mm) from the first observation made by Robison *et al*. (2014) and the hatchling size (ML = 28 mm) described by Voight & Drazen (2004). We used the size of ripe eggs (not the egg size at hatching), since the size of ripe eggs is available for more species, and can be obtained from mature pre-spawning females. Spawned eggs and egg size at hatching are still unknown for most cephalopod species, except for the unique case of *G. boreopacifica* where egg growth until hatching was observed using a submersible.

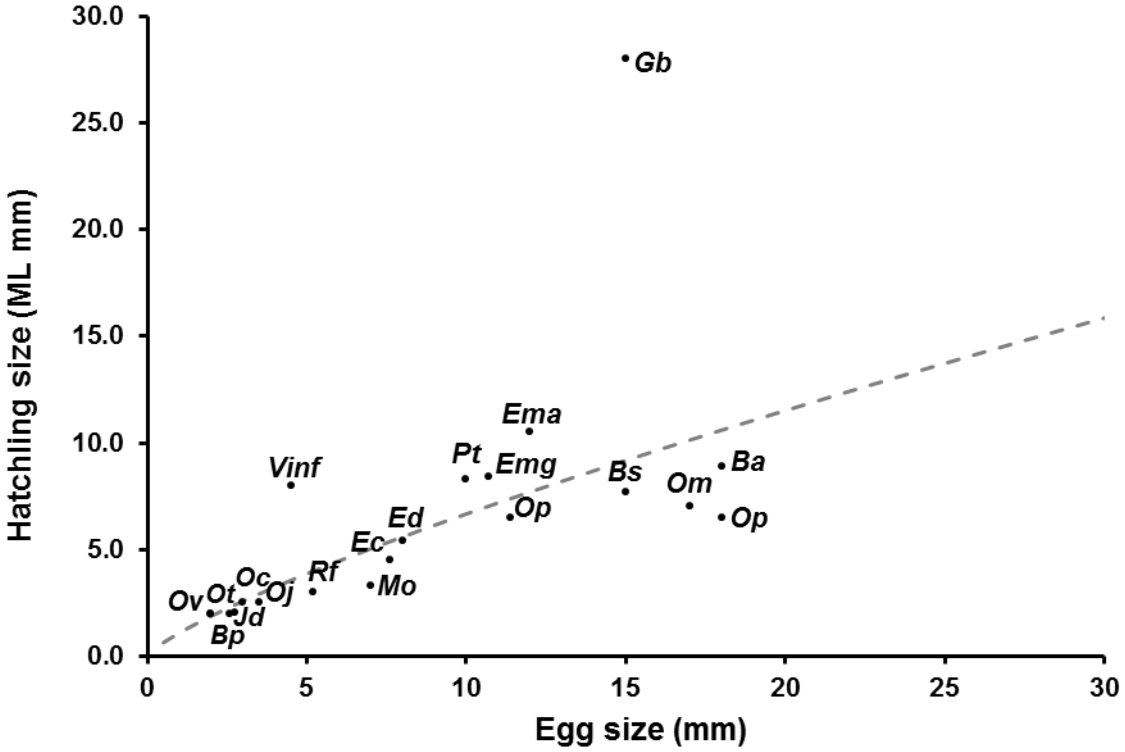

Fig 1. Relationship between egg size (*EgS*) and hatchling size (*HTs*) for 20 species of octopods and *Vampyroteuthis infernalis*. The fitted model (*HTs* = $1.086EgS^{0.788}$; r^2 = 0.70; *F* = 42.7, *df* = 18, *p* < 0.01) is influenced by the large hatchlings of *Graneledone boreopacifica* (Gb) (mantle length [ML] = 28 mm, Voight & Drazen 2004) at the top of the plot. For the full species names, see Table 1.

When hatchling weight was not available the instantaneous growth rate (*G)* was calculated using derived data from the fitted relation between hatchling size and hatchling weight (*HTw*) of 10 benthic incirrate octopods species (Fig 2). This relationship was best described by the power function: $HTw = 0.0018HTs^{2.174}$ (r^2 = 0.93; $p < 0.001$), where hatchling weight is in g and hatchling size is the mantle length in mm.

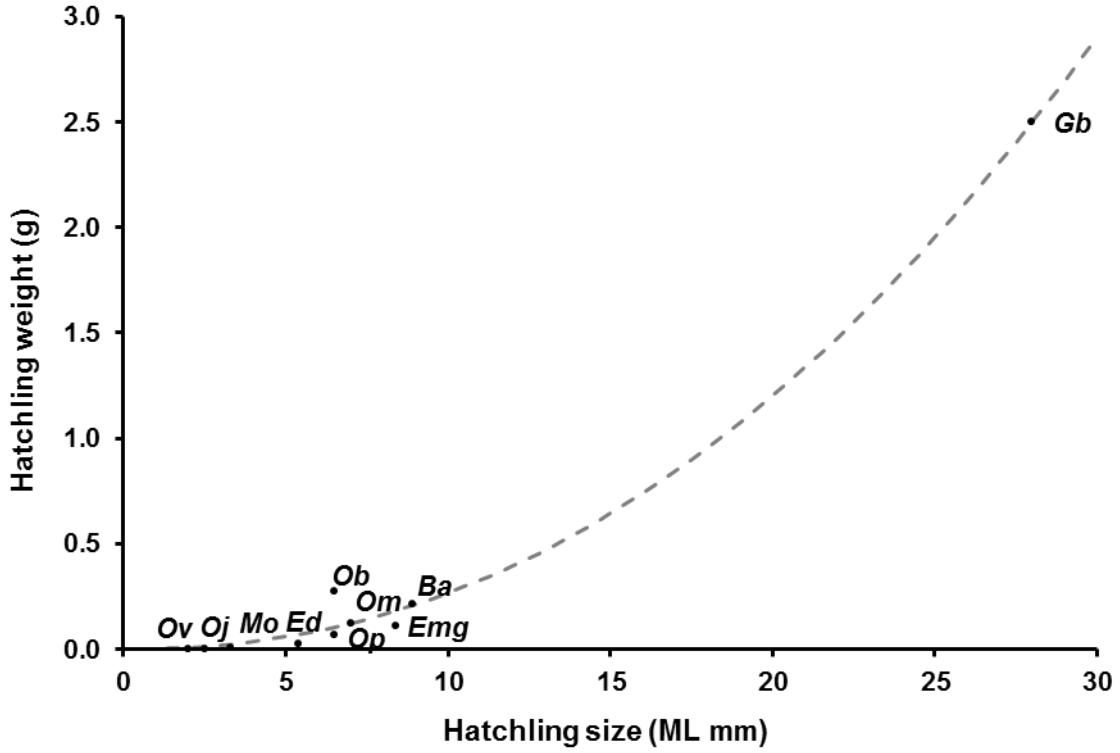

Fig 2. Relationship between hatchlings size (*HTs*) and hatchling weight (*HTw*) for 10 species of incirrate octopods. The power curve ($HTw = 0.0018HTs^{2.174}$, $r^2 = 0.93$) is influenced by the extremely large and developed hatchling of *Graneledone boreopacifica* (*Gb*) at the right end of the plot. For the full species names, see Table1.

1.3.6 Regression analyses

We tested for correlations between age at maturity and growth rates, average temperature and species' depth of occurrence (Table 1). Our analyses were limited by the fact that population depth distributions/ranges are known, but not where the highest population density occurs. We therefore tested the relationship between age at maturity and growth rates with the minimum, average (midpoint) and maximum depths of the species' known distribution range.

When the conditions for independence, normality and equal variance were met, data were $\log_{10}$ transformed to correct for non-linearity in the data and to prevent heterogeneity of variances. For the pelagic octopods *J. diaphana* and *B. pygmaea*, no information on weight at maturity was available. Therefore, these species were not included in the correlation between instantaneous growth rates (*G*) and environmental variables. We also analysed the relationships between age at maturity, growth rates and average lifetime temperature using the Arrhenius plot and by fitting linear regressions:

$$ln\ Age\ _{or}\ ln\ G = a - b(1000/T) \quad (3)$$

where T is the average lifetime temperature in degrees Kelvin, *a* is the intercept and *b* is the slope of the linear regression. The Arrhenius plot aims to test how rates of biological processes function during an increase in temperature (Peck *et al*. 2007, Peck 2016). All parameters were estimated using the functions *lm* and *nls* from the package *'stats'* in the software R 3.5.0 (R Development Core Team 2018).

1.4 Results

1.4.1 Life-history data in relation to environmental variables

Temperature

A strong negative relationship between average lifetime temperature and age at maturity was found (Table 2). Adjusting the power function ($Age = 3039Temp^{-0.61}$) heuristically showed that age at maturity increases 1 order of magnitude in species living at temperatures below 5°C (Fig 3A). Based on environmental temperature, octopods may be divided into 3 groups: species inhabiting waters between 10° and 25°C, pelagic deep-sea octopods and *Bathypolypus arcticus* clustering around 5°C, and benthic deep-sea and polar octopods living in temperatures below 3°C (Fig 3A). Since for some species age at maturity was calculated from their embryonic development time estimates (based on temperature), some of the data points are overlapping. This occurred for the pelagic species *Japetella diaphana*, *Bolitaena pygmaea* and *Vampyroteuthis infernalis* (4.5°C); the benthic Antarctic species *Adelieledone polymorpha*, *Pareledone aequipapillae* and *Pareledone turqueti* (1°C), and for *Graneledone* spp. (see Table 1).

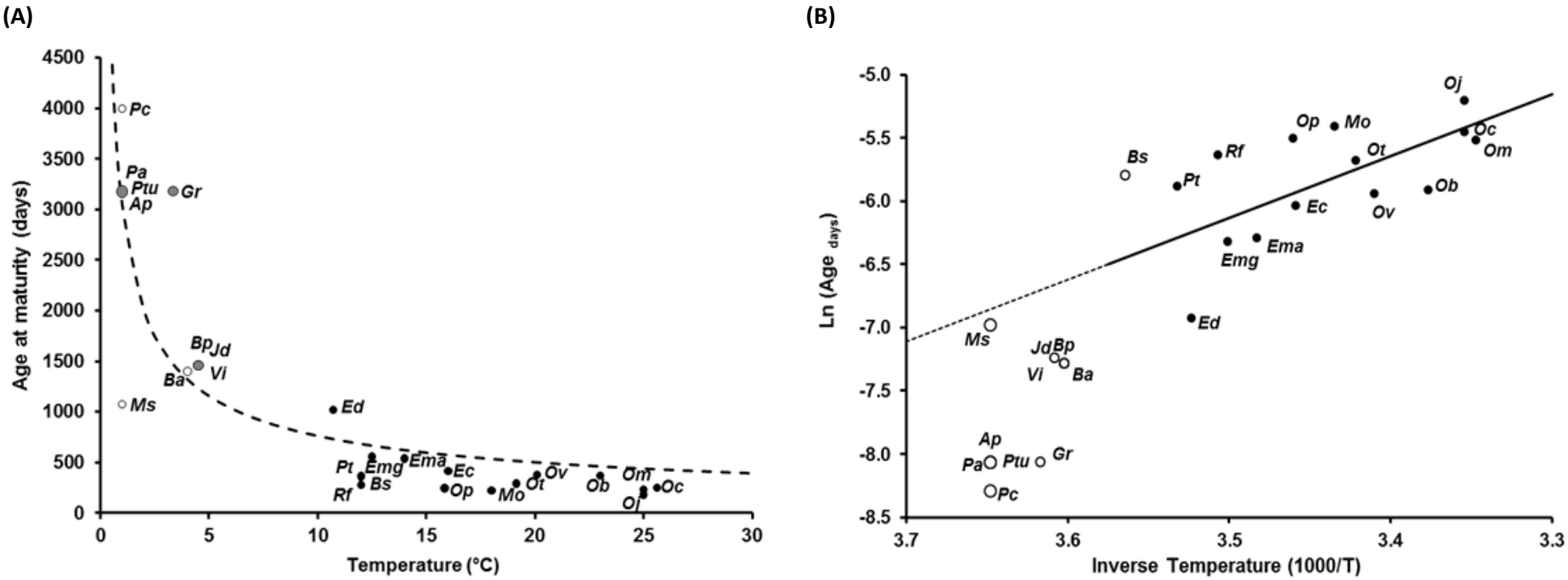

Fig 3. Relationship between age at maturity and habitat temperature for 25 octopod species and *Vampyroteuthis infernalis*. (A) The dashed line shows the predicted model ($Age = 3039Temp^{-0.61}$, $r^2 = 0.86$). White (open) circles represent deep-sea and Antarctic octopods (< 5°C), black circles represent temperate and tropical species (>5°C). Grey circles indicate overlapped data points. (B) Arrhenius plot of the data used in (A). Solid line is the relationship for species living in temperatures above 5°C ($LnAge = 5.97 - 3.42(1000/T)$; $r^2 = 0.30$, $F = 5.58$, $df = 14$, $p < 0.05$). The dashed line represents an extension of the relationship to temperatures below 5°C. For full species names, see Table 1.

The Arrhenius plot for the same data revealed that temperate and tropical species follow a linear relationship with the inverse temperature values (Fig 3B). These species are distributed along the regression line, while cold water species (<5°C) aggregate far from the extrapolated fitted model (*Ln Age* = 5.97 – $3.42\left({}^{1000}/_{T}\right)$; r^2 = 0.30, *F* = 5.58, *df* = 14, *p* < 0.05). This pattern suggests that age at maturity in cold-water octopods cannot be solely predicted by environmental temperature. The values show that the time to reach maturity is delayed beyond the predicted effects of temperature on biological systems. While temperate and tropical species typically reach maturity in less than 2 yr, deep-sea species are predicted to mature after 4 yr, and Antarctic species inhabiting waters around 0°C after 8 yr of age (Fig 3A).

While lifespan tends to increase as habitat temperatures decrease, the growth rates (*G*) are positively correlated with temperature with species growing faster at temperatures between 15° and 25°C (Fig 4A). An Arrhenius plot of the instantaneous relative growth rates showed a strong linear relationship for tropical and temperate species (Fig 4B). As observed in the age at maturity plot, the estimated growth rates for polar and deep-sea species are much lower than the extrapolation of the relationship for temperate and tropical species (*Ln G%* = 17.95 – $4.90\left({}^{1000}/_{T}\right)$; r^2 = 0.47, *F* = 11.4, *df* = 14, *p* < 0.005).

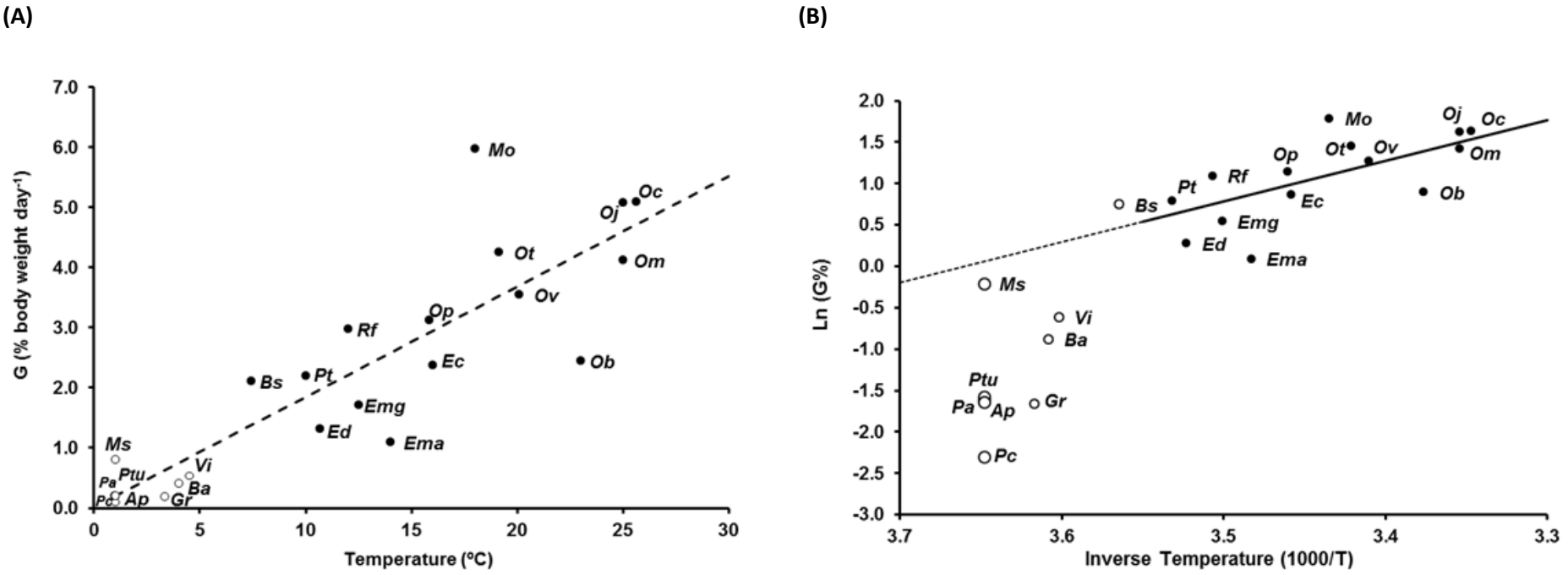

Fig 4. Growth rates and average lifetime habitat temperatures. (A) Instantaneous growth rate G (% body weight day^{-1}) versus average habitat temperature of 24 octopod species (excluding bolitaenids). White circles represent deep-sea and Antarctic octopods (<5°C), black circles represent temperate and tropical species (>5°C). The dashed line is the adjusted linear regression [$G = 0.01 + 0.18Temp$, $r^2 = 0.77$]. (B) Arrhenius plot from same data. Solid line is the relationship for species living above 5°C ($Ln\ G\% = 17.95 – 4.90(1000/T)$; $r^2 = 0.47$, $F = 11.4$, $df = 14$, $p < 0.01$). The dashed line represents an extension of the relationship to temperatures below 5°C. For the full species names, see Table 1.

Depth of occurrence

The correlations between depth of occurrence (minimum, midpoint and maximum), age at maturity and growth rates were examined (Table 2). Although the regressions have non-zero slopes, the data were highly scattered around the fitted models (Fig 5). The model which best described the relationship uses the maximum depths of occurrence and it predicts ages at maturity close to the observed data (age estimated from increments in beaks, stylets and eye-lenses) for species inhabiting shelf and slope depths shallower than 100 m (Fig 5). Five of the 7 *Octopus* spp. and the Maori octopus *Macroctopus maorum* were placed close to the regression line, maturing before 2 yr. The long-lived Antarctic species, typically distributed over depths shallower than 500 m, were outliers and biased the correlation. The environmental conditions (e.g. temperature, hydrostatic pressure) for the Antarctic shelf and slope species can be similar to those experienced by cephalopods inhabiting the deep sea (>500 m) (Clarke 2003). The number of increments in the stylets of the deep-sea octopus *Bathypolypus sponsalis,* which occurs in water depths between 900 and 1250 m in the Northeast Atlantic region, suggests that its lifespan is less than 1 yr (Barratt & Allcock 2010). All other species inhabiting water depths deeper than 1000 m were estimated to reach maturity after 3 yr (Fig 5).

Table 2. Summary of the regression results for the comparisons. *G*: Growth rate (%); Temp: average lifetime temperature (°C); Depth (m); Age (days); W: weight at maturity (g); SHSI: species hatchling size index.

Comparison	Model	Correlation (r^2)	*F* Stat (*df*)	*P* value
Lifespan				
Age at maturity *vs* Temp	$Age = 3039Temp^{-0.61}$	0.86	96.8; *df* = 23	$p < 0.01$
Age at maturity *vs* Depth (max.)	$\text{Log}[Age] = 1.70 + 0.46\text{Log}[Depth]$	0. 47	20.3; *df* = 23	$p < 0.01$
vs Depth (average)	$\text{Log}[Age] = 1.89 + 0.49\text{Log}[Depth]$	0. 45	18.7; *df* = 23	$p < 0.01$
vs Depth (min.)	$\text{Log}[Age] = 2.64 + 0.22\text{Log}[Depth]$	0. 38	13.8; *df* = 23	$p < 0.01$
Growth rates				
G vs Temperature	$G = 0.01 + 0.18Temp$	0.77	70.0; *df* = 21	$p < 0.01$
G vs Depth (max.)	$G = 7.51 - 2.2\text{Log}[Depth]$	0.64	35.9; *df* = 21	$p < 0.01$
vs Depth (average)	$G = 6.69 - 2.08\text{Log}[Depth]$	0.62	33.9; *df* = 21	$p < 0.01$
vs Depth (min.)	$G = 3.0 - 1.0\text{Log}[Depth]$	0.44	16.2; *df* = 21	$p < 0.01$
Arrhenius Plot relationship				
Age at maturity *vs* Temp	$Ln\ Age = 5.97 - 3.42(1000/T)$	0.30	5.6; *df* = 14	$p < 0.05$
G vs Temp	$Ln\ G\% = 17.95 - 4.90(1000/T)$	0. 47	11.4; *df* = 14	$p < 0.01$
Size vs lifespan - Do large *octopods live longer?*				
Age at maturity *vs* weight at maturity	$\text{Log}[Age] = 3.22 - 0.15\text{Log}10[W]$	0.06	1.3; *df* = 21	$p =$ 0.27[a]
Do species with large hatchlings live longer?				
Age at maturity *vs* SHSI	$Age = 26.45 + 177.69SHSI$	0.52	24.2; *df* = 23	$p < 0.01$

[a]Accept Ho slope = 0, i.e. correlation not significant

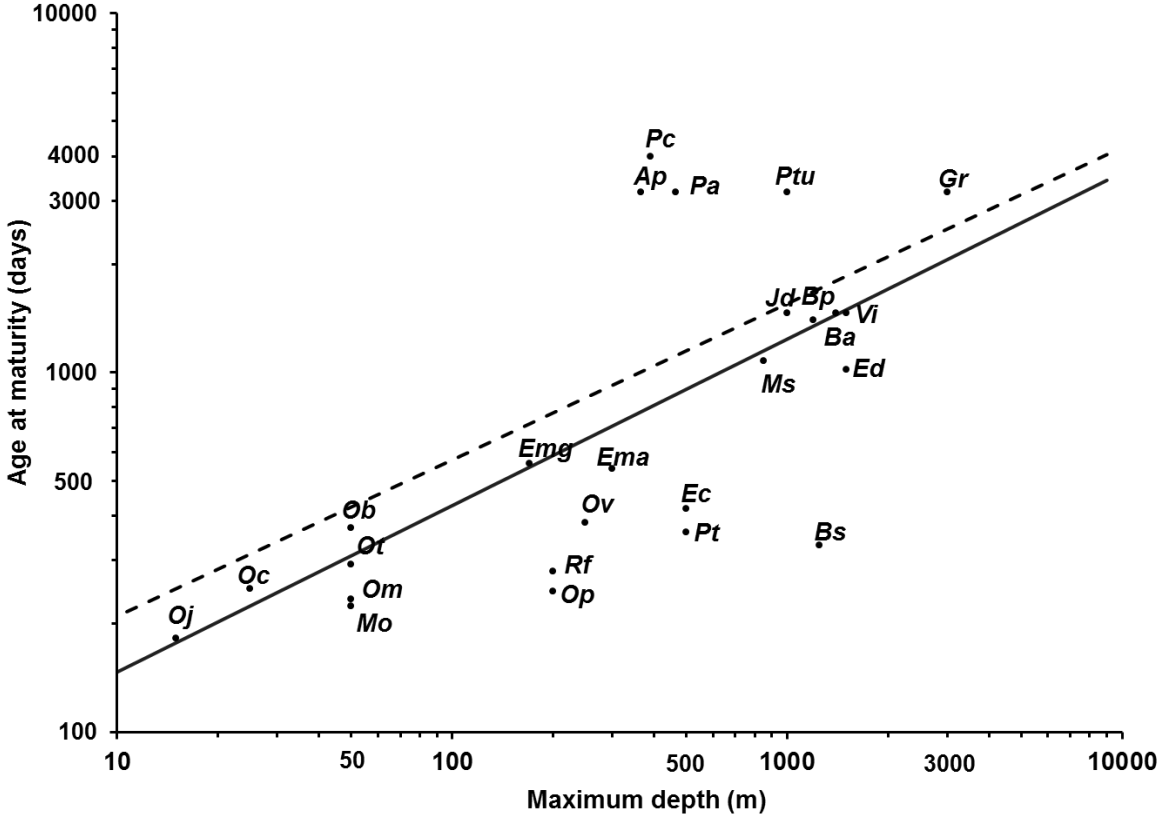

Fig 5. Age at maturity related to maximum depth of occurrence for 26 octopod species, including *Vampyroteuthis infernalis*. Tick marks are displayed on a logarithmic scale. The continuous line shows the adjusted linear model (*Log*[*Age*] = 1.70 + 0.*46Log[Depth]*, r^2 = 0.47). The dashed line represents the same relationship but using average depths. For the full species names, see Table 1

The regression analysis between instantaneous growth rates and maximum depth of occurrence shows again that species which occur in deep waters (>500 m), exhibited growth rates similar to species living in polar regions (*G* < 2% body weight d^{-1}) (Fig 6). For subtropical and temperate species inhabiting waters shallower than 500 m, growth rates ranged from 2.4 to 6% body weight d^{-1}. Other shallow water octopods exhibited intermediate growth rates ranging between 4 and 5% (Fig 6). Of the species that occurred deeper than 1000 m, *B***.** *sponsalis* presented the fastest growth rates (*G* = 2.1%)**,** while the deep-sea genus *Graneledone* (*G*. *boreopacifica* and *G*. *macrotyla*), which can occur down to 3000 m, exhibited very low growth rates (*G* = 0.2%).

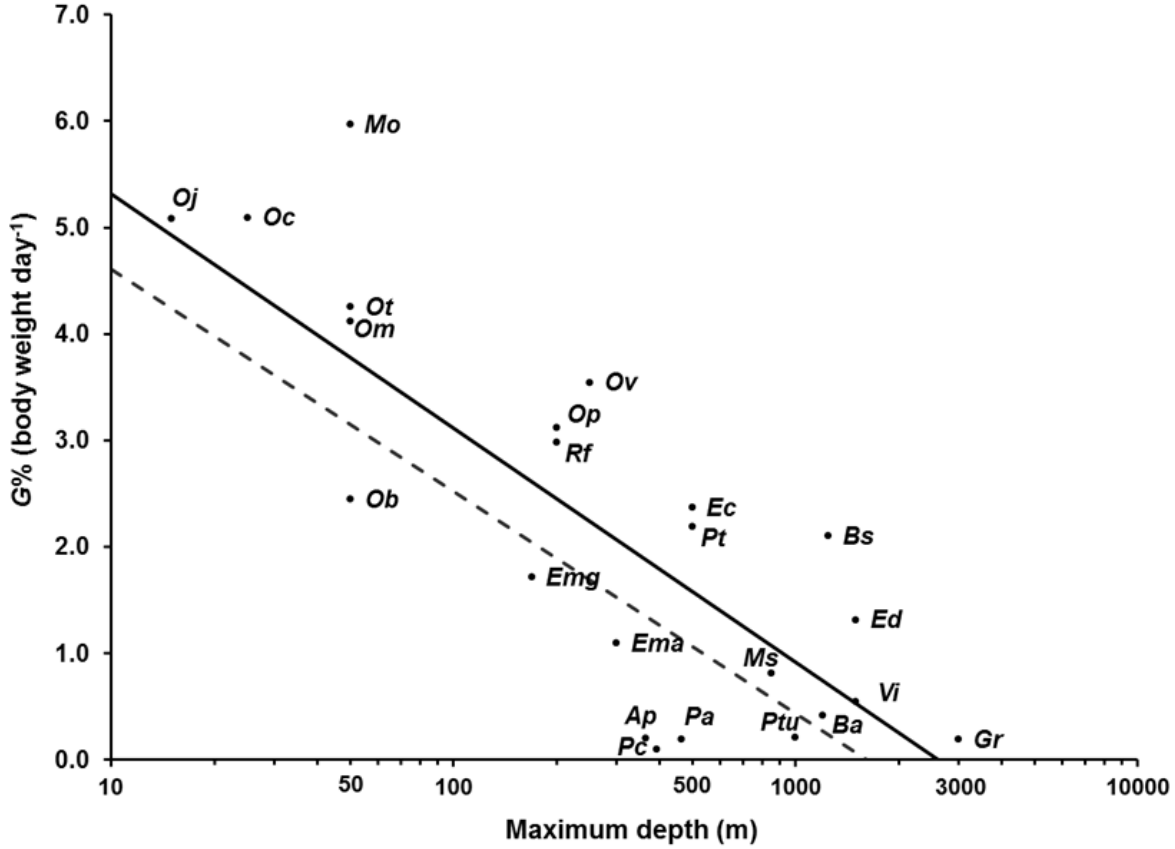

Fig 6. Relationship between growth rates (*G*) and depth (m) for 24 octopod species. Tick marks are displayed on a logarithmic scale. Linear regression between growth rate *G* (% body weight d^{-1}) and maximum depth of occurrence ($G = 7.98 – 2.37Log[Depth]$, $r^2 = 0.68$). The continuous line represents the relationship using maximum depth of occurrence, while the dashed line represents the relationship using average depths. For the full species names, see Table 1.

1.4.2 Body weight and age at maturity

Our analyses covered a wide range in body sizes. The weight at maturity of the largest octopod species (*Enteroctopus dofleini*), was 500 times larger than the body weight of the smallest octopus, the Atlantic pygmy octopus *Octopus joubini* (Table 1). The slope of the correlation between age and weight at maturity ($Log[Age] = 3.22 – 0.15Log[W]$; $r^2 = 0.06$; $p > 0.27$) did not differ from 0 (Table 2). In our data set *E. dofleini*, *Megaleledone setebos* and *Macroctopus maorum* were the only species that weighed on average more than 5000 g at maturity, and only *M. maorum* attains this average body weight in less than 1 yr. The majority of the octopods with expected longevities exceeding 3 yr weighed less than 1000 g at maturity.

1.5 Discussion

1.5.1 Growth rates, size at maturity and temperature

In this study we tested the hypothesis that lifespan in octopods is inversely correlated with temperature and depth distribution. We used published data from 26 species of octopods inhabiting water temperatures from -2 to 25°C, and found that species inhabiting colder waters have increased lifespans compared to warm-water species. The data analysis illustrates the diversity of life history strategies, even within the same order of cephalopods, and a tight coupling between longevity and environment. For ectotherms such as cephalopods, temperature controls the time required for embryonic development and maturation, which can consequently increase longevity (Table 2). Our results suggest that octopods living in polar and deep-sea habitats (<5°C) reach maturity after 3 yr (~1000 d, Fig 3A). The total lifespan of species for which the females brood the eggs after spawning can be considerably longer. The duration of the brooding time largely depends on the temperature at which the eggs have to develop.

Wood & O'Dor (2000) found a positive correlation between size and age at maturity. They noted that octopods typically mature at lower weights and have reduced growth rates compared to squids and cuttlefish. We did not find a significant correlation between age and weight at maturity (Table 2). The absence of such a correlation indicates that, at least for our data set, the octopods' body weight cannot predict age. This may be attributed to the fact that the body weight data set used was not evenly represented. We included few large species and many of the small species lack validation of the estimated lifespan (e.g. Antarctic octopods). For example, annual species of the genus *Octopus* reach maturity at an average weight of 2 kg within 1 yr, while most of the smaller Antarctic and deep-sea pelagic species (<1 kg) are predicted to reach maturity after 3 yr. Based on the available literature, the giant Antarctic octopus *Megaleledone setebos* (~8 kg) and the giant Pacific octopus *Enteroctopus dofleini* (16 kg) are estimated to reach maturity after 3 yr. However, *M*. *setebos* can grow up to 25 kg (Piatkowski *et al*. 2003) and *E*. *dofleini* was reported to reach 71 kg (Cosgrove & McDaniel 2009), suggesting that these species can grow faster or live longer. Another large octopus which inhabits the cold waters of the meso- and bathypelagic zone is the benthopelagic giant deep-sea octopus *Haliphron atlanticus*. Females of this species brood their eggs, can grow up

to 75 kg (O'Shea 2004) and prey on gelatinous zooplankton (Hoving & Haddock 2017). Although there are no age estimates for *H. atlanticus*, the lifespan may be comparable to that observed in *E*. *dofleini* or *M*. *setebos*.

Intermediate-size octopod species from tropical and temperate waters are fast-growing, with growth rates of 3–6% body weight d^{-1} (Forsythe & Van Heukelem 1987, Iglesias *et al*. 2014). However, those species can exhibit high intra-specific variation in growth, with animals that hatch with similar sizes (ML = 2–3 mm) reaching very different maturity sizes under the same temperature regimes. This is due to highly flexible life history strategies where size-at-maturity depends on genetics, environmental temperature, food availability and potentially a combination of these factors. Similar trends have been published for squids (e.g. Hoving *et al*. 2013, Arkhipkin *et al*. 2015, Takahara *et al*. 2017). Octopods from cold waters mature late and are typically of small to medium size, with the exceptions of *M. setebos* and *E. dofleini*. For *Bathypolypus sponsalis*, a small species usually inhabiting waters around 12°C (Quetglas *et al*. 2001), the predicted daily growth rate of 2% is 4 times higher than that estimated for *B*. *arcticus* (*G* = 0.41%) living at average temperatures of 7°C. This illustrates the interspecific variation in growth rates of congeneric octopods, but the observed differences in growth rates may not solely be driven by environmental differences, but also by uncertainties related to age estimation. Age estimations based on the quantification of stylet increments in *B*. *sponsalis* suggest an annual lifespan with fast growth rates (2%); however, the periodicity of stylet increment deposition for this species is not validated (Barratt & Allcock 2010). Lower growth rates were measured for *B*. *arcticus* in experimental studies (Wood 2000, Wood & O'Dor 2000). The daily growth rate of 0.4% that was measured for *B*. *arcticus* reared at 7°C corresponds with a growth rate of 0.1% for the Antarctic octopod *Pareledone charcoti* kept at 0°C (Daly & Peck 2000), and fits in the life history trends observed in our study. For all octopods inhabiting water temperatures lower than 10°C, the instantaneous growth rates were less than 1%. The polar species *P. charcoti* (*G* = 0.1%) grows 50 times slower than the similar-sized tropical pygmy octopus *O. joubini* (*G* = 5%).

From an ecophysiological perspective, temperature seems to be the most important physical environmental factor affecting growth performance of ectotherms (Atkinson 1996), a trend that was confirmed in our analysis. For cephalopods, lower temperatures result in reduced growth rates and can increase size-dependent

mortality by predation (Wood 2000). At higher temperatures animals can grow fast, potentially to avoid predators, but mortality due to starvation will tend to increase if food is scarce and growth rates can be reduced if the temperatures surpass the optimum tolerance values (Rodhouse *et al*. 2014). The Arrhenius plots that test how rates of biological processes change during a temperature increase; revealed that the effects of the temperature on age at maturity and growth rates for polar and deep-sea species are slowed beyond the predicted relationship fitted for temperate and tropical species (Figs. 3B & 4B). The results presented here for octopods are congruent with those found for brooding in gastropod molluscs and growth rates in echinoids (Peck *et al*. 2007, Peck 2016). Evidence suggests that the low water temperatures of polar and deep-sea regions may exert a constraint on protein synthesis, and this may delay the processes involved in embryonic development and growth (Peck 2016).

1.5.2 Octopod age estimates derived from embryonic duration

Sizes of eggs and hatchlings are significant predictors of lifespan in cold water octopods. The relationship between SHSI and the maximum adult size was positive, indicating that longer lifespans are expected for species producing large eggs and hatchlings with mantle lengths beyond 10–15% of the adult's size (Table 2). Among the incirrate octopods, egg sizes vary from 0.8 mm in the pelagic *Argonauta* spp. to more than 40 mm in the giant Antarctic octopus *M. setebos* (Boletzky 1992, Allcock *et al*. 2003). In all incirrate octopod species, egg brooding covers the embryonic development partially or completely. We used this aspect of the life cycle to estimate the longevity of some octopod species based on Boletzky's (1987) hypothesis which states that embryonic development in cephalopods may vary from a few percent to as much as one third of the lifespan. Following this hypothesis, we conservatively assumed that brooding time in cold water octopods is one third of the total lifespan (Robison *et al* 2014). Although this hypothesis remains to be tested, our calculations suggest a brooding period between 25 and 35% of the age at maturity for 7 octopus species (Table 1), corresponding well with Boletzky's assumptions. For the temperate species *Octopus pallidus,* which produces some of the biggest eggs in this genus, embryonic development can last up to 50% of the estimated age at maturity. Species living at temperatures above 15°C were estimated to have shorter brooding times lasting less than 20% of the age at maturity.

Following the above trends we may carefully infer brooding times for other cephalopod species. Vampire squid spawn batches of 10–100 eggs, where the spawning of each batch is separated by a gonadal resting phase (Hoving *et al* 2015). Unlike incirrates, vampire squids release individual eggs of 4–5 mm that are believed to drift in the water column between 600 and 1500 m (Pickford 1949, Hoving *et al*. 2015), but spawning vampire squids have never been observed in their natural habitat. Gonatid and bathyteuthid squids spawn eggs of similar dimensions (>3 mm) as vampire squid do, and brood their egg masses in their arm crown (Seibel *et al*. 2005, Bush *et al*. 2012). Seibel *et al*. (2005) estimated that female squids *Gonatus onyx* brood eggs up to 9 mo in water temperatures of 1.7–3.0°C in the bathypelagic zone. Oogenesis in vampire squid takes place between 2° and 7°C, and the hatchling size is about 8 mm ML (Young & Vecchione 1999). This means that after spawning, the egg nearly doubles in size from 4.5 mm to ~8.0 mm before hatching occurs. Based on the egg development model in Robison *et al* (2014), the development time of vampire squid eggs is approximately 2 yr. Pelagic incirrate bolitaenids live and brood eggs at depths similar to those at which vampire squids occur (Young 1972). By brooding, the female provides protection to the eggs and is able to control under which hydrographic conditions (depth, temperature, salinity, and oxygen concentration) the eggs will develop, which may reduce offspring mortality. By being released into the water column, vampire squid eggs may suffer relatively high mortality rates, e.g. as a result of predation, fouling and hypoxia. On the other hand, the relatively high potential fecundity of up to 20,000 eggs (Hoving *et al*. 2015) may compensate for high mortality.

The environmental conditions under which the embryos of incirrate octopods develop may influence the hatchlings' early mode of life (benthic or pelagic) (Villanueva *et al*. 2016) and likely the life-cycle duration (Wood 2000, Robison *et al*. 2014). Although egg development times are extremely long for certain octopod species inhabiting water temperatures below 5°C (e.g. *G*. *boreopacifica*, 53 mo at 3°C; Robison *et al*. 2014, Purser *et al* 2016), such long development times are not uncommon for other marine invertebrates at high latitudes (Hain & Arnaud 1992, Peck *et al*. 2006). Hain & Arnaud (1992) investigated the reproductive strategy of 66 molluscan species from the Weddell Sea (Antarctica), and found that the intracapsular development of the gastropod species *Torellia mirabilis* and *Trophon scotianus* lasted up to 25 mo, a strategy that resulted in well-developed benthic

juveniles. The authors concluded that gastropods from the Weddell Sea produce eggs that are 4 times larger and that the embryonic development can be up to 30 times longer than in related temperate gastropods (Hain & Arnaud 1992). The large egg cases of the Antarctic nudibranch *Bathydoris hodgsoni* are estimated to require up to 10 yr of embryonic development, which result in one of the largest mollusc hatchlings known to date (Moles *et al*. 2017). Investigations on the reproductive biology of various different taxa (Annelida, Arthropoda and Mollusca) inhabiting the deep seafloor revealed that brooding, large egg sizes and increased longevities are common specializations of the fauna living in these environments (Young 2003), which agrees with our results discussed here. Overall it seems evident that egg and larval development in Antarctic marine ectotherms is considerably slower than in temperate species (Peck 2002, Peck *et al*. 2006). This phenomenon is known as Thorson's rule (Mileikovsky 1971), which states that egg sizes and embryonic development duration in benthic ectotherms is positively correlated with latitude and depth. Thorson's Rule has been tested and confirmed for several taxa, including octopods (Ibáñez *et al*. 2018).

The methods presented in this study are limited by the high intraspecific variability of sizes at maturity in cephalopods, temperature regimes and depth distribution ranges which all may confound the analysis. Another limitation of the method used here is that the life history traits of the analysed species may not be phylogenetically independent and require independent contrasts to eliminate phylogenetic bias (Harvey & Pagel 1991). Although the phylogenetic comparative methods are assumed as the most statistically correct, they also can present drawbacks. Cornwell & Nakagawa (2017) warned that inferring life history traits and environmental conditions of ancestral forms can induce more uncertainties in regression analysis in certain cases. Three main sources of uncertainty are pointed out by the authors: (1) wrong phylogenetic tree reconstruction based on limited data – where misplacement of species can result in wrong distances of the tree branches and the values in the tree nodes. This is particularly important for our dataset since the phylogeny of the Octopoda is still not fully resolved (Strugnell *et al*. 2014); (2) trait uncertainty – traits may not be representative for the species as a whole, and frequently they are obtained from few observations or single populations; and (3) model uncertainty – the Brownian motion model of evolution may not apply in all cases. Due to these uncertainties we consider further evolutionary analyses of our

examined parameters beyond the scope of this paper since first more basic data on life history and octopod phylogeny is required.

Nevertheless, the theoretical approach using representatives of the order Octopoda living in a great variety of environments provides the first trends on how habitat characteristics may impact the life cycle of cold-water octopods, allowing the development of hypotheses and research questions for future research. New research should focus on obtaining life-history information (growth rate, age at maturity, longevity, spawning strategy) on abundant cold-water octopods which can be integrated in the models developed here. The advancement of deep-sea observational technology to study cephalopods in their natural habitat will further allow us to obtain new information on cephalopod biology, life cycles and behaviour (Hoving *et al*. 2014). The investigation of life cycle length and growth of cephalopods inhabiting cold water environments becomes important to evaluate how resilient these species can be against ongoing and future habitat disturbances (e.g. deep-sea mining, fisheries, and predator removal).

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Authors’ contributions

R.S., H.J.H and U.P. designed the study; R.S. collected the data, performed the analyses and wrote the first manuscript draft. All authors edited and revised the manuscript.

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Chapter 2

Life histories of Antarctic incirrate octopods (Cephalopoda: Octopoda)

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2.1 Abstract

As a general trend in the life history of marine organisms, species inhabiting cold waters have reduced growth rates and increased lifespans. Studies based on egg sizes and brooding times of deep-sea and polar octopods support this hypothesis, but empirical data on growth are still scarce. To test the hypothesis that octopods inhabiting cold waters (< 3 °C) live longer than temperate and warm water species, this study investigated size-at-age, maturation and growth rates in incirrate Antarctic octopods. Octopod age was estimated via the interpretation and quantification of beak growth increments, which in shallow water octopods have been validated to be formed on a daily basis. Specimens from the families Megaleledonidae (*Adelieledone* spp., *Pareledone* spp. and *Megaleledone setebos*) and Enteroctopodidae (*Muusoctopus rigbyae*) were collected on the shelf and slope regions off the Antarctic Peninsula during a cruise in 2012. Examined specimens included early juveniles to animals in advanced maturity. The total number of growth increments ranged from 192-599 in *Pareledone aequipapillae* (body mass [BM] 2-109 g), 182-431 in *Pareledone charcoti* (BM 5-124 g), 98-906 in *M*. *setebos* (BM 10-6000 g) and 207-425 in *M*. *rigbyae* (BM 24-256 g). After the cruise, eleven specimens of *P*. *charcoti* were kept alive in captivity for more than 12 months and these animals had 219-364 growth increments, suggesting that increment formation in this species takes longer than one day. The complex population structure (size, age and maturity range) of the specimens that were captured during a relatively short time, the number of beak increments quantified, and the preliminary validation observations indicate that Antarctic octopods do not deposit increments daily, and may have lifespans exceeding 3 years. These findings corroborate the general trend that cold water molluscs have a longer lifespan than their warm water relatives.

2.2 Introduction

The marine benthic invertebrate fauna of the Southern Ocean is highly diverse and adapted to the harsh environmental conditions of high latitudes (Clarke & Johnston 2003). Antarctic invertebrates evolved in such a way that these organisms are able to cope with extremely low temperatures and highly seasonal productivity (Clarke 1988, 2003). The permanently cold water (-2 to 3°C) slows down physiological processes in Southern Ocean marine ectotherm invertebrates, resulting in increased lifespans in echinoderms, molluscs and crustaceans compared to their temperate relatives (Peck *et al.* 2006, Vogt 2012, Moss *et al.* 2016). As an example, the crangonid shrimp *Notocrangon antarcticus* (Pfeffer, 1887) from the Weddell Sea lives in a temperature range of -2 to 0.4°C with a lifespan of 8 to 10 years, while *Crangon crangon* (Linnaeus, 1758) from the North Sea lives for approximately three years at temperatures of 7 to 16°C (Vogt 2012). Life history theory predicts that animals with increased lifespans mature relatively late in life and invest more energy in fewer but larger offspring. These traits increase survival in the hatchlings, and compensate for the mortality risks of a longer embryonic and juvenile period (Stearns 2000).

Many benthic molluscs from the Southern Ocean follow the trend described above and typically invest in the production of well-developed benthic juveniles, avoiding the high mortality rates experienced by planktotrophic larvae which are common in temperate species (Clarke 1992, Peck *et al.* 2006). Gastropod species from the Weddell Sea can produce eggs that are four times larger than those of their temperate relatives, whilst embryogenesis can be 30 times as long, with some species taking as much as 25 months to hatch (Hain & Arnaud 1992). The large egg cases (52 mm) produced by the Antarctic nudibranch *Bathydoris hodgsoni* (Eliot, 1907) are estimated to require up to 10 years for their complete embryonic development. The hatchlings of this species are 29 mm and among the largest hatchlings of molluscs (Moles *et al.* 2017).

Southern Ocean cephalopods are also known to produce fewer and larger offspring than their tropical and temperate counterparts (Collins & Rodhouse 2006). Benthic incirrate octopods are the most diverse cephalopod group in the region, comprising 27 of the 54 cephalopod species identified to date (Xavier *et al.* 2018). The majority of octopod species endemic to the Southern Ocean belong to the family

Megaleledonidae, which also contains deep-sea taxa that occur in other ocean regions (Strugnell *et al.* 2014). On the shelf regions of the Antarctic Peninsula and South Shetland Islands, the most common octopod species are from the genus *Pareledone* (10 species), the genus *Adelieledone* (three species) and the giant Antarctic octopus *Megaleledone setebos* (Robson, 1932) (Allcock *et al.* 2001, 2003ab, Strugnell *et al.* 2017).

Antarctic octopods are important components in the diets of demersal fishes, southern elephant seals and Weddell seals (Daneri *et al.* 2000, 2012, Stevens *et al.* 2014). However, knowledge about their feeding ecology and trophic positions are still preliminary (Daly & Rodhouse 1994, Piatkowski *et al.* 2003, Negri *et al.* 2016). Reproductive studies of Antarctic octopods have revealed that all species produce large eggs, which likely produce benthic crawling hatchlings that are adult miniatures (Kuehl 1988, Hochberg *et al.* 1992, Kubodera & Okutani 2004, Barratt *et al.* 2008, Laptikhovsky 2013). The embryonic development is estimated to be extremely slow, lasting from months to years and potentially takes up a substantial part of their lifecycles (Boletzky 1987, 1992, Robison *et al.* 2014).

Lifespans of Southern Ocean octopods are unknown, but their life history traits (egg size, fecundity, environmental temperature, adult size) suggest that they live longer than the typical 1-2 years found in temperate and tropical forms (Schwarz *et al.* 2018). Age estimates in cephalopods can be derived from the quantification of growth increments in hard body parts including statoliths, stylets and gladii (*i.e.* vestigial shells), eye lenses and chitinous beaks (Arkhipkin *et al.* 2018). Statoliths are calcareous structures typically used to age squids and sepioids, and which growth increments have been validated to be deposited daily (Arkhipkin & Shcherbich 2012). Octopod statoliths lack discernible growth increments and are not suitable for age estimates (Lombarte *et al.* 2006, Perales-Raya *et al.* 2010). However, growth increments in beaks and stylets were validated to be formed on an approximately daily basis in octopods living in water temperatures > 17 °C (Hermosilla *et al.* 2010, Rodríguez-Domínguez *et al.* 2013, Perales-Raya *et al.* 2014, 2018). The increment periodicity in hard body parts of Antarctic octopods is unknown. Assuming a daily deposition, the quantification of growth increments in *M. setebos* stylets suggested a lifespan of about four years (Barratt & Allcock 2010). *Pareledone* species have small and delicate stylets which are difficult to prepare for age estimation, and *Adelieledone* lack stylets (Allcock 2005). Total lifespan in *Pareledone charcoti*

(Joubin, 1905) is unknown, but the few specimens kept under laboratory conditions survived up to 21 months and had average daily growth rates of 0.11%, the slowest growth rate ever measured for octopods (Daly & Peck 2000).

While age estimation using stylets is prone to limitations, all cephalopod species have chitinous beaks which also have been used for age determination in squids and octopods (Arkhipkin *et al.* 2018). In most cephalopod species the beaks are easy to extract, store and manipulate, and can provide ecological information even when retrieved from predator stomachs (Clarke 1986). To assess if Antarctic octopod species have longer lifespans than tropical and temperate counterparts the present study investigates size-at-age, growth rates, longevity and maturation of Antarctic incirrate octopods using the quantification of growth increments in beaks.

2.3 Materials and Methods

2.3.1 Collection of specimens

Species analyzed in this study belong to the Southern Ocean incirrate octopod family Megaleledonidae (two *Adelieledone* spp., eight *Pareledone* spp. and *Megaleledone setebos*) and the family Enteroctopodidae (*Muusoctopus rigbyae*; Vecchione, Allcock, Piatkowski & Strugnell 2009) which all inhabit the continental shelf and slope areas of the Antarctic Peninsula (Allcock 2005, Vecchione *et al.* 2009). The specimens were during a demersal fish trawl survey in March and April 2012 (RV POLARSTERN cruise PS79-ANTXXVIII/4) (Noever & Piatkowski 2012). The bottom trawls were conducted at depths between 50-480m north of the Antarctic Peninsula and in the vicinity of the South Shetland Islands (Fig 1). Detailed information on the water temperatures, nets used, fishing depths and geographic position of trawling stations is available in the POLARSTERN 2012 expedition PS79 report (Lucassen 2012). During the cruise, approximately 400 net collected specimens of the species *Pareledone charcoti* were kept alive in aquaria on board and transported to the Alfred Wegener Institute (AWI, Bremerhaven, Germany). The animals were kept alive in the laboratory from the time of their capture during March-April 2012 until April 2013 (Oellermann 2015, Oellermann *et al.* 2015). Eleven of these specimens were used to compare the number of growth increments in the beaks with the time they were kept in captivity, in order to estimate the periodicity of beak increment deposition. The remaining octopod specimens were immediately

frozen after sorting and brought to GEOMAR Helmholtz Centre for Ocean Research (Kiel, Germany) where they were stored at -40 °C.

In addition, two male specimens of *M. setebos* (BM 1598 - 3858 g) collected during RV POLARSTERN southern Weddell Sea cruises in 2013/2014 (Knust & Schröder 2014) and 2015/2016 (Schröder 2016) were also analyzed. These two animals were donated to our project by the AWI. The upper beak from a large male specimen of *M. setebos* collected in February of 1989 during a cruise to Halley Bay and Kapp Norvegia (Arntz *et al.* 1990) was also examined. For this animal only the length was recorded (DML = 210 mm) and its weight was estimated using the allometric equation $BM=0.0009DML^{2.937}$, which resulted in a body mass of ~ 6000 g (Piatkowski *et al.* 2003). The three males were included in order to estimate age in large specimens (> 1000 g), since most animals collected in 2012 weighed less than 500 g.

All octopods examined in this study are non-endangered species. All applicable international or institutional guidelines for the care and use of animals were followed. The research was conducted in accordance with the guidelines and ethics of the German law. Sampling during the research expeditions was conducted in accordance with the Commission for the Conservation of Antarctic Marine Living Resources (CCAMLR) (Lucassen 2012). Experiments involving animal husbandry at AWI were approved by the veterinary inspection office (Senatorin für Wissenschaft, Gesundheit und Verbraucherschutz, Bremen, Germany) under the Permit Number AZ: 522-27-11/02-00 (93) (Oellermann *et al.* 2015).

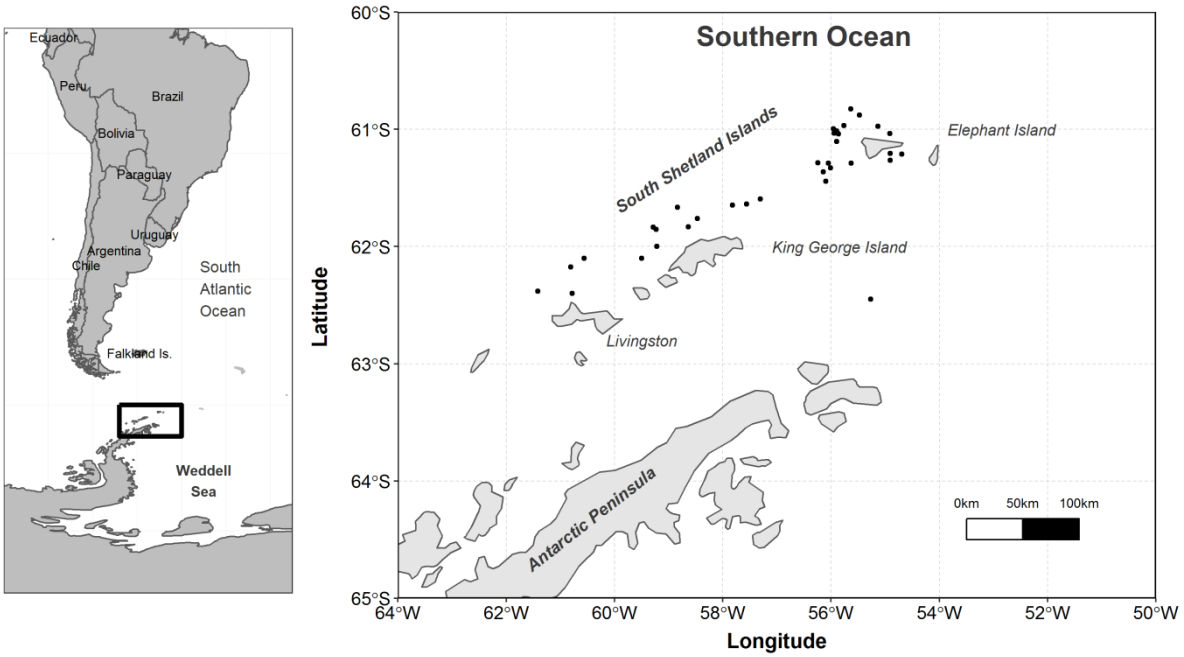

Fig 1. Map of study region. Black dots represent bottom trawl stations (RV POLARSTERN cruise PS79) in the vicinity of the South Shetland Islands in March-April 2012. Sampling depths and biological parameters of octopods collected are summarized in Table 1.

2.3.2 Summary of specimens

The frozen specimens were thawed in the laboratory and dorsal mantle length (DML, mm), ventral mantle length (VML, mm), total length (TL, mm), body mass (BM, grams), sex and maturity stages were recorded. The species were identified following the descriptions published by Allcock et al. (Allcock *et al.* 2003a, 2003b, Allcock 2005) and Vecchione et al. (2009). Due to the poor condition, five octopod specimens could not be identified to species level and were not included in the analyses. In total 882 incirrate Antarctic octopods were examined (Table 1).

Table 1. Octopod species examined including biological parameters (Sex, DML=dorsal mantle length, BM=body mass, N=specimen numbers, Depth=sampling depth ranges, and Beaks read=number of beaks that were read to quantify growth increments).

Species	Sex	DML (mm) min-max (**avr**)	BM (g) min-max (**avr**)	Depth (m) min-max (**avr**)	N	Beaks read
Adelieledone piatkowski	♂	62 - 65 (63)	56 - 92 (77)	428	3	0
	♀	68 - 75 (72)	104 - 114 (109)	428	2	0
Adelieledone polymorpha	♂	28 - 84 (57)	6 - 132 (63)	155 - 438 (291)	66	6
	♀	20 - 97 (55)	4 - 201 (63)	100 - 466 (291)	83	5
	und	26 - 26 (26)	4 - 4 (4)	207 - 207 (207)	1	0
Adelieledone sp	♀	19	1	250	1	0
Megaleledone setebos	♂	27 - 230 (82)	11 - 9760 (1005)	146 - 880 (321)	32	27
	♀	27 - 160 (58)	13 - 2860 (240)	123 - 428 (307)	29	23
	und	210	~6000	782	1	1
Muusoctopus rigbyae	♂	47 - 88 (69)	41 - 320 (136)	62 - 425 (295)	36	23
	♀	31 - 89 (64)	24 - 337 (133)	62 - 475 (318)	23	15
Pareledone aequipapillae	♂	21 - 78 (44)	4 - 109 (31)	100 - 428 (323)	137	82
	♀	17 - 64 (43)	2 - 79 (31)	92 - 428 (307)	75	51
Pareledone albimaculata	♂	30 - 36 (32)	12 - 18 (15)	275 - 387 (331)	4	0
	♀	33 - 49 (41)	21 - 86 (54)	241 - 241 (241)	2	0
Pareledone aurata	♂	29 - 56 (44)	5 - 47 (32)	127 - 387 (231)	18	2
	♀	15 - 52 (37)	3 - 41 (23)	100 - 250 (179)	13	0
Pareledone charcoti	♂	27 - 57 (44)	5 - 49 (28)	62 - 197 (110)	92	15
	♀	18 - 77 (49)	3 - 124 (46)	62 - 387 (108)	84	29
Pareledone cornuta	♂	21 - 53 (37)	3 - 56 (29)	170 - 332 (235)	24	0
	♀	22 - 56 (35)	9 - 60 (27)	127 - 334 (241)	13	0
Pareledone felix	♂	22 - 67 (52)	5 - 77 (42)	146 - 438 (310)	38	8
	♀	21 - 70 (46)	2 - 100 (39)	100 - 438 (251)	23	7
Pareledone subtilis	♂	38 - 55 (49)	11 - 43 (34)	146 - 197 (162)	6	0
	♀	31 - 31 (31)	9 - 9 (9)	197 - 197 (197)	1	0
Pareledone turqueti	♂	6 - 100 (56)	2 - 613 (112)	100 - 475 (248)	40	7
	♀	21 - 116 (60)	6 - 561 (112)	93 - 438 (231)	30	4
	und	30 - 30 (30)	12 - 12 (12)	339 - 339 (339)	1	0
Pareledone sp	♀	43 - 43 (43)	32 - 32 (32)	279 - 279 (279)	1	0
	und	-	19 - 34 (28)	264 - 475 (353)	3	0
Total					**882**	**305**

♂ - male; ♀- female; *und* – sex undetermined.

2.3.3 Reproductive aspects

Comprehensive descriptions of the reproductive system and biology of the species examined in this study (*e.g*. morphology and development of gonads, spermatophores and oocytes) have been provided in earlier studies and will not be discussed in detail (Kuehl 1988, Daly 1996, Allcock *et al.* 2003b, Allcock 2005, Barratt *et al.* 2008, Laptikhovsky 2013). Typically, Southern Ocean octopods have a reproductive system in an advanced stage of development at very small sizes (DML > 20 mm). Males are easily recognizable due to the large sized hectocotylus on the

tip of their third right arm. Although females lack distinct external features, juveniles have distinct oviducts and ovaries.

Maturity stages were assessed following an adaptation of the 6 maturity stage scales defined by Vecchione et al. (2009) and Daly (1996). For juvenile males in stage II, the whole reproductive system (spermatophoric organ and testis) was weighed. In maturing males (stages III-VI) in which the spermatophoric complex and the testis could be distinguished, the two parts were weighed separately. Spermatophores number and length (SpL) from 160 individuals were also recorded. To examine females for traces of mating activity, the mantle cavity and oviducts were inspected for the presence of spermatophores or spermatangia. The complete female reproductive system (*i.e.* ovary, oviducal glands, and oviducts) was removed and weighed. The diameters of the ovaries and the oviducal glands were measured. The ovary was cut open and the oocytes were counted, measured and weighed. The ovaries were also examined for signs of previous spawning events, such as empty follicles and resorbed oocytes. The presence of small oocytes was checked using a dissecting microscope (6.3 to 25x).

For 107 female specimens all the oocytes in the ovary were examined. The oocytes from immature ovaries are spherical (diameter ≤ 2 mm) and unimodal in size. For 47 females with developing and mature ovaries, all oocytes were separated from the connecting epithelial stalks and measured. Oocytes < 4 mm were measured using a stereomicroscope eyepiece graticule; Oocytes > 4 mm were measured using a digital calliper. All weights and measurements were recorded to the nearest 0.1 mg and 0.01 mm. The gonadosomatic index was calculated as: GSI = (GW/BM)*100%, where GW is the total reproductive apparatus weight and BM is the animal's total weight in grams. Animals were considered mature when GSI > 8% and vitellogenic oocytes were larger than 10 mm (Daly 1996, Allcock 2005).

2.3.4 Beak extraction

Thawed specimen beaks were dissected by making an incision on the buccal mass using scissors. The remaining tissues and mucus on the beaks were removed by hand under running tap water and by using cleaning wipes. When necessary, the beaks were submerged for some minutes in diluted sodium hypochlorite (NaCL) and cleaned again. The beaks were soaked in 70% ethanol (to kill bacteria), placed in vials containing distilled water and stored in a refrigerator (4-7°C) until further analysis.

2.3.5 Age estimation analysis using beaks

Procedures for preparing and quantification of beak growth increments followed the methods described by Hernández-López et al. (2001) and Perales-Raya et al. (2010). Beaks were sectioned using scalpels or razor blades in order to obtain two symmetrical halves (Fig 2).

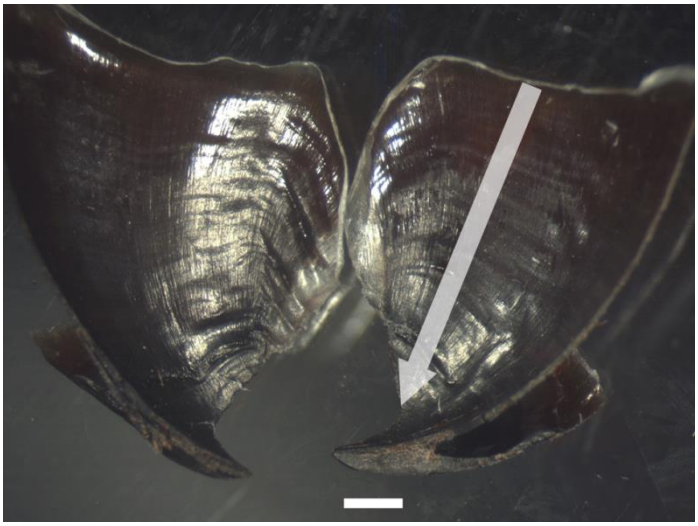

Fig 2. The two halves of a sectioned upper beak from a female of *Pareledone aequipapillae*. Scale bar = 1 mm; the arrow indicates the counting axis.

The growth increments were best visualized and counted in the internal part of the upper beaks' lateral walls surface (LWS). The LWS were examined using a combination of epi-illumination (gooseneck illuminator) and transmitted light. In dark pigmented regions of the beak only reflected light was suitable for increment visualization. The magnification chosen for growth-increment counting ranged between 32 and 50x. Beak hoods were removed for better positioning of the lateral wall surfaces on microscope glass slides and in order to improve light transmission.

Beaks were placed with their inner surface facing upwards. To prevent dehydration and cracking during increment visualization, the portion of the beak in contact with the microscope slide was kept hydrated with distilled water. Increments were observed via a stereo microscope camera (Leica MZ 9.5) and several (overlapping) photographs were taken in order to cover the entire LWS area (Fig 3). Increment counts and measurements of the distances between growth increments (increment width, µm) were performed with image analysis software (Image Pro Insight®). When increment visibility on the anterior region near to the rostrum was poor, an extrapolation was made. The distance of the extrapolated region was divided by the average width of the last counted visible increments.

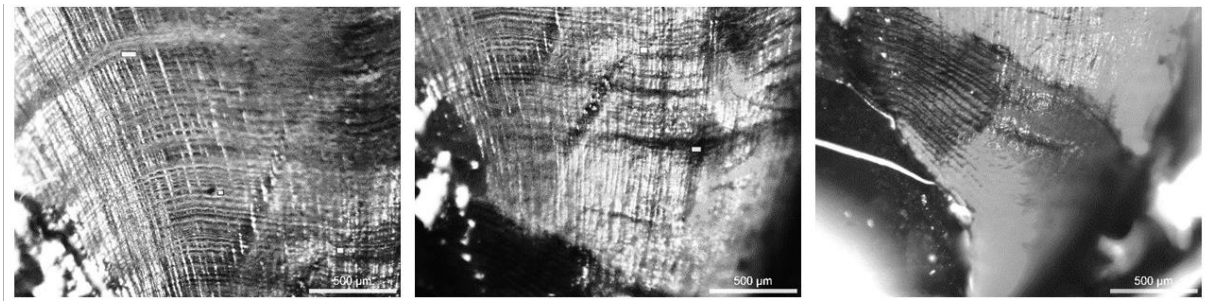

Fig 3. Growth increments in the inner surface of the upper beak lateral wall from a juvenile female stage II of *P. charcoti* (DML 43, BM 24 g). Left, posterior region close to the border; Center, medial region of the beak; Right, anterior region close to the rostrum where the first increments were formed, showing check marks in the shoulder region (magnification 50x).

2.3.6 Species selected for age estimation

Four species; *M. setebos*, *M. rigbyae*, *Pareledone aequipapillae* (Allcock, 2005) and *P. charcoti* were selected for age estimation through the observation of growth increments in the upper beak LWS. These species occurred in relatively high numbers (N=266) and in a variety of sizes and maturity stages.

2.3.7 Precision of growth increment counting

To obtain the total number of increments in each beak, two counts were performed by a single reader (the first author). The counts were conducted at different occasions. When the increment number differed by more than 10%, a third count was made. If the percentage of agreement did not increase after the third count the beak was excluded from the analysis. Increment count precision was assessed with the coefficient of variation (CV) (Chang 1982) and the average percent error (APE) (Beamish & Fournier 1981). These terms result from the equations:

$$\mathrm{CV_j} = 100\% \times \frac{\sqrt{\sum_{i=1}^{R} \frac{\left(X_{ij} - X_j\right)^2}{R-1}}}{X_j} \qquad (1)$$

$$\mathrm{APE_j} = 100\% \times \frac{1}{R} \sum_{i=1}^{R} \frac{|X_{ij} - X_j|}{X_j} \qquad (2)$$

where X_{ij} is the i^{th} age determination of the j^{th} beak, X_j is the mean age estimate of the j^{th} beak, and R is the number of times each octopus beak was aged. When two age estimates were possible, the age was assumed to be the mean value between counts.

2.3.8 Growth increment periodicity

For some octopod species, the periodicity of growth increment formation has been validated to occur on a daily basis (Rodríguez-Domínguez *et al.* 2013, Perales-Raya *et al.* 2014). To test the hypothesis that growth increments are produced on a daily basis in Antarctic octopods, the beaks of specimens of *P. charcoti* that were kept in captivity were examined. Eleven female *P. charcoti* captured in the 2012 RV POLARSTERN cruise were maintained in aquaria on board and transported to the AWI. The animals were kept in tanks with a re-circulating aquaculture system at 0°C from late March 2012, and were fed one to two times per week with both live and frozen *Crangon crangon* shrimps until they were sacrificed in mid-April 2013 after approximately 12 months (Oellermann 2015). Administration of chemical tracers like oxytetracycline or calcofluor (Hermosilla *et al.* 2010, Perales-Raya *et al.* 2014) in order to evaluate beak increment periodicity was not possible. Unfortunately, we learned about the AWI animals survival time only after Oellermann's publication in 2015 (Oellermann *et al.* 2015). The carcasses of the octopods were donated to our study and we compared the approximate husbandry time (~ 12 months) plus the time in the wild, with the number of growth increments in their upper beak LWS.

2.3.9 Growth

In order to compare growth rates derived from beak growth-increment with those from husbandry experiments (Daly & Peck 2000), we simulated that increments were formed daily. The instantaneous growth rate *G* (% body mass d^{-1}) was calculated for each 60-day interval by sex using the equation (Forsythe & Hanlon 1988, Hu *et al.* 2016):

$$G = \frac{ln(S_2) - ln(S_1)}{(t_2 - t_1)} . 100 \qquad (3)$$

where S_1 and S_2 were the average BM (g) at the beginning (t_1) and end (t_2) of time interval, respectively.

For the species in which a representative dataset was available, allometric relationships between dorsal mantle length and body mass [$BM = aDML^b$], and between body mass and growth-increment number (g*inc*) [$BM = a ginc^b$] were heuristically fitted using least squares method. All statistical analyses were performed using R version 3.5 and RStudio (Rstudio Team 2015).

2.4 Results

We examined a total of 882 octopod specimens from twelve species belonging to four genera. The most numerous species were *Pareledone aequipapillae* and *Pareledone charcoti* which accounted for 44% of the specimens examined (appendix S2 Fig 1). The number of species per haul ranged from 1 to 12 ($\overline{x}$ = 4 spp.). The species often overlapped in their spatial and bathymetric distributions in the northern part of the South Shetland Islands, around the Elephant Island and northwest of the Antarctic Peninsula (S2 Fig 2), and the spatial patterns were similar to those found in previous studies (Allcock *et al.* 2003ab, 2007, Allcock 2005, Vecchione *et al.* 2009).

2.4.1 Size distribution and maturity

A wide range of sizes and maturity stages were captured for all species and for both sexes (Fig 4). In ♀ *P. charcoti*, ♀ *Megaleledone setebos*, ♀ *M. rigbyae* mantle length and body mass frequency distributions did not follow a normal Gaussian distribution, but a multimodal distribution (S2 Fig 3). Therefore, size differences between sexes were evaluated using the non-parametric Wilcoxon-Mann-Whitney *U*-test. Differences in body mass between sexes were observed only in *P. charcoti* (Table 2). For this species the Wilcoxon's test indicated that females (BM $\overline{x}$ = 45.9 g) weighed on average more than males (BM $\overline{x}$ = 28 g; U = 817, $p < 0.001$).

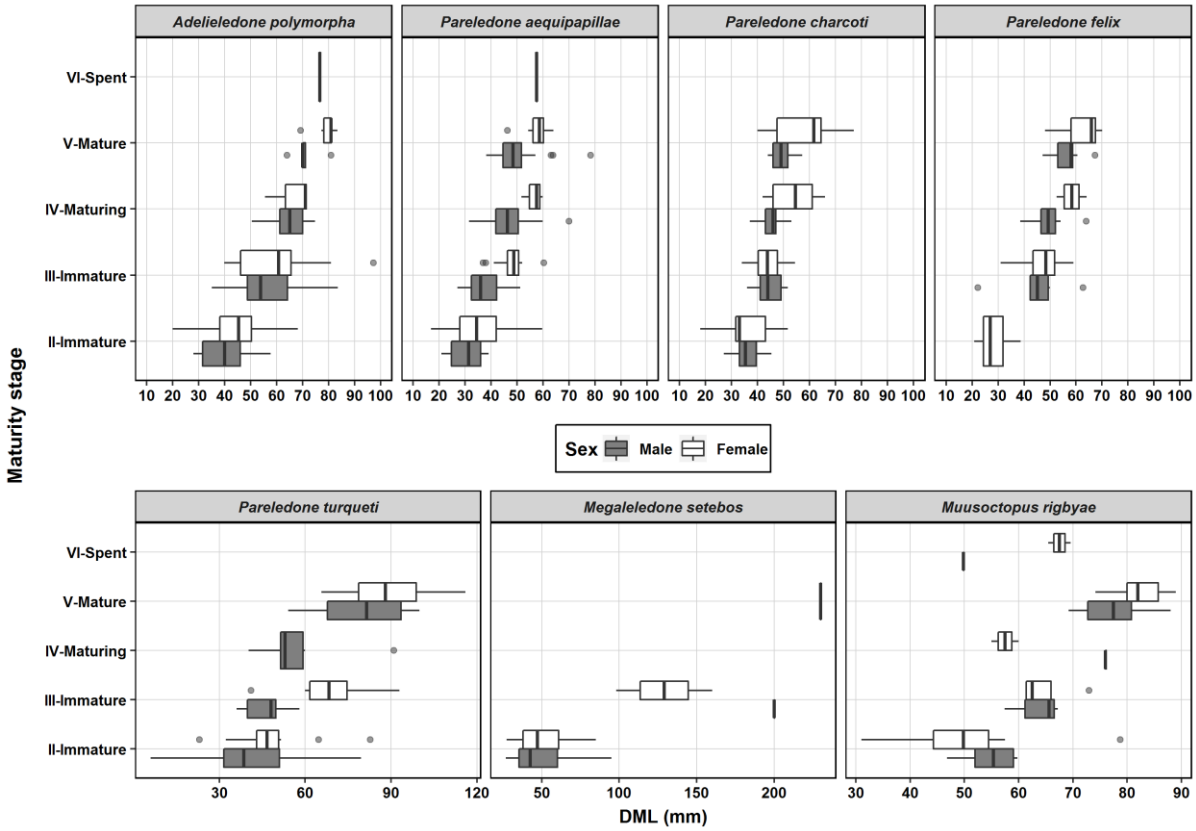

Fig 4. *Size distribution at different maturity stages of the most numerous species. Grey circles are outliers.*

Table 2. Results of Mann–Whitney–Wilcoxon test for differences of size or weight between sexes.

Species	Variable	Means $\bar{x}$ (♂ | ♀)	Mann-Whitney test *U*	*p*-value
A. polymorpha	DML (mm)	56.8 | 55.3	2972	0,37
	BM (g)	62.6 | 63.3	2816	0,77
M. rigbyae	DML (mm)	68.9 | 64.3	482	0.29
	BM (g)	136 | 133	432	0.78
M. setebos	DML (mm)	75.3 | 58.1	481	0.49
	BM (g)	890 | 240	471	0.59
P. aequipapillae	DML (mm)	44.4 | 42.7	5488	0.41
	BM (g)	30.6 | 30.7	5189	0.90
P. charcoti	DML (mm)	44.0 | 48.7	3013	0.01
	BM (g)	28.0 | 45.9	2132	*< 0.001**
P. felix	DML (mm)	51.5 | 45.7	537	0.13
	BM (g)	42.0 | 39.6	481	0.52
P. turqueti	DML (mm)	56.5 | 59.6	560	0.64
	BM (g)	112 | 112	572	0.74

Five specimens of *Adelieledone piatkowski* (Allcock, Hochberg, Rodhouse & Thorpe, 2003) were collected during one haul at 428 m, east of King George's Island. The two females were heavier than the three males (Table 1) and all were immature. For *Adelieledone polymorpha* (Robson, 1930), no sexual size dimorphism was observed (Fig 5).

The largest size range was observed in *M*. *setebos*, with the majority of animals being juveniles (BM 10 to 500 g) and only a few maturing specimens (BM > 1000 g) (Fig 5). *Pareledone turqueti* (Joubin, 1905) and *M*. *rigbyae* were of intermediate size and could reach more than 300 g, but most individuals weighed less than 150 g. Other *Pareledone* species were typically of small size (BM < 200 g) and weighed on average less than 100 g (Fig 5). The relationship between dorsal mantle length and body mass was similar between sexes (appendix supplement S2 Table 1). In *P. charcoti*, males weighed less than females of the same size.

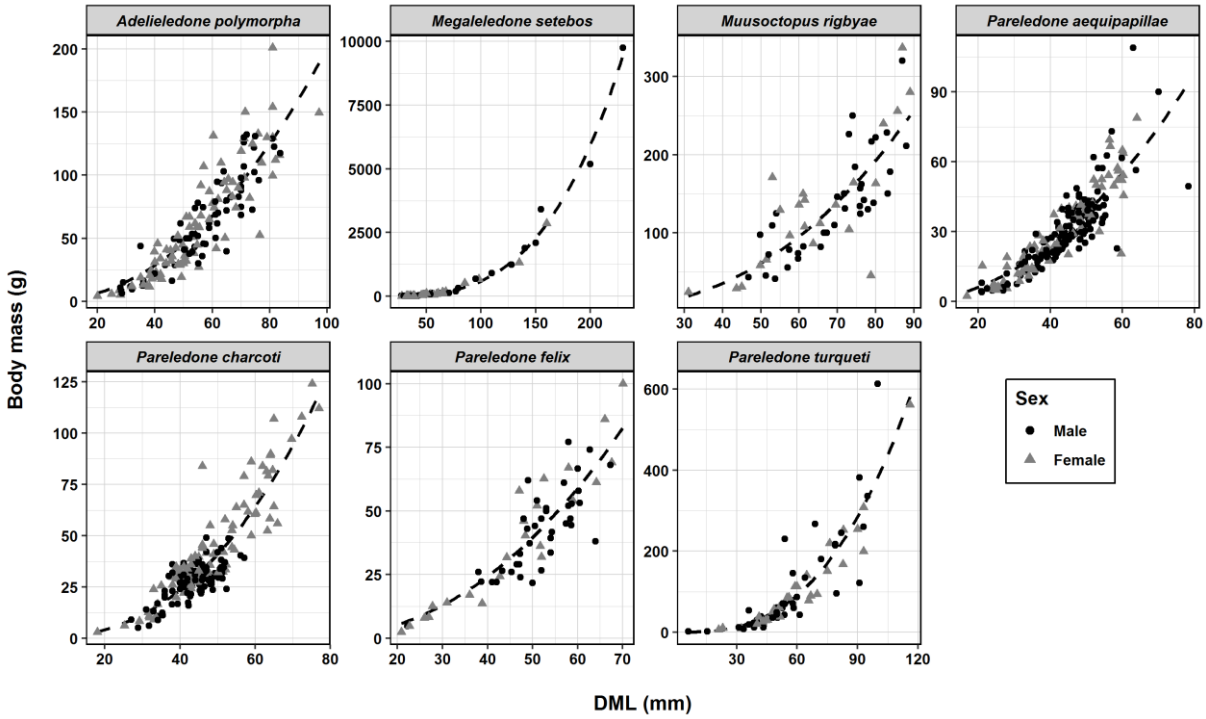

Fig 5. Length-weight relationship of the seven most numerous octopod species. Scale of axes differs between species due to size differences.

2.4.2 Description of the beaks

The beaks (upper and lower) of the five most abundant species follow the typical octopodid beak morphology (Fig 6, except *Adelieledone* spp. and *M*. *setebos*). All species have robust, darkly pigmented upper and lower beaks with a well-developed hood and curved rostrum with a blunt tip (Fig 6B). The upper beaks of *M. setebos* and *A. polymorpha* possess a round and shallow projection where the rostrum was supposed to occur, and the crests lack curvature (Fig 6B and E). In *Adelieledone* the lower beaks have a characteristic rostrum which is very small, round, sharp and oriented in a straight or upward angle (Fig 6B).

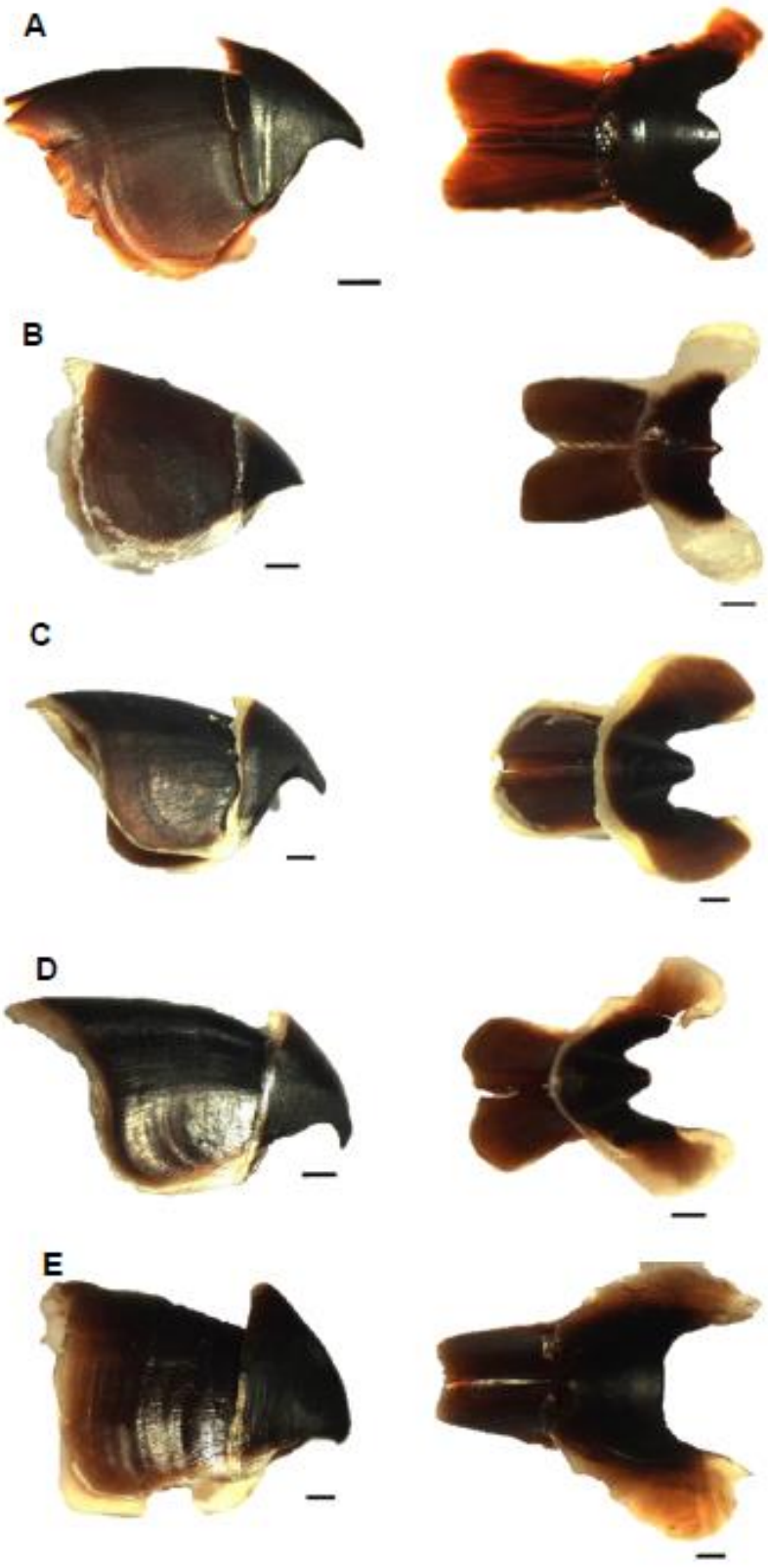

Fig 6. Upper (left) and lower (right) beaks of the five most abundant species of Antarctic octopods analyzed in this study. **A** - *Muusoctopus rigbyae*; **B** - *Adelieledone polymorpha*; **C** - *Pareledone aequipapillae*; **D** - *Pareledone charcoti*; **E** – *Megaleledone setebos*. Scale bars represent 1 mm.

The increment width (W_{inc}) varied between different species and regions of the beak LWS. In *M*. *setebos* growth increment width ranged from 10 to 78 µm (mean W_{inc} = 40 µm), in *M*. *rigbyae* they are of intermediate size (10 - 49 µm; $\bar{x}$ = 25 µm), in *P*. *aequipapillae* they ranged from 8 to 42 µm ($\bar{x}$ = 19 µm), while in *P*. *charcoti* they ranged from 6 to 47 µm ($\bar{x}$ = 21 µm). The medial and posterior regions, which are the most recently formed, had higher variability when compared to the anterior region near to the rostrum (Fig 7).

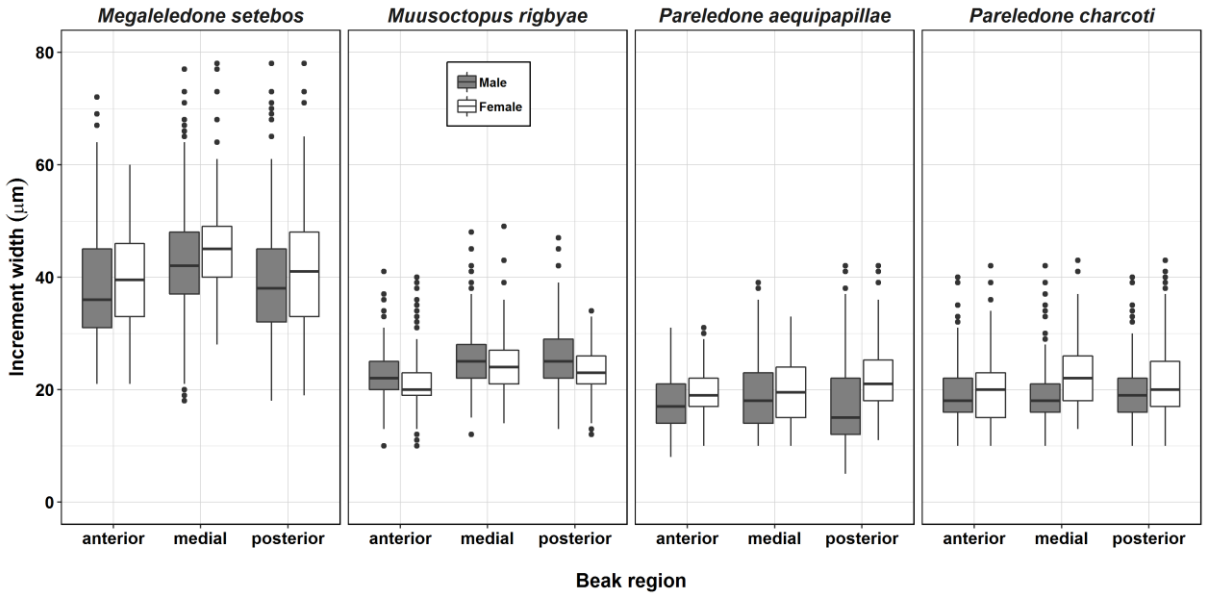

Fig 7. Increment width in different regions of the lateral walls of the octopus beaks analyzed. The anterior region indicates the oldest portion close to the rostrum while the posterior region bear recent formed increments near to the edge of the crest.

2.4.3 Precision of age estimates

For 229 beaks (38 *M*. *rigbyae*, 51 *M*. *setebos*, 96 *P*. *aequipapillae*, 44 *P*. *charcoti*) the counting of growth increments was performed twice. An age bias plot was produced to identify systematic bias between two sets of age estimates from the same beak, using the first count as the reference estimate (appendix supplement S2, Fig 4). The precision between two counts was higher in *M*. *setebos* compared to other species (CV and APE < 2%). For *P*. *charcoti*, the number of growth increments was higher in the second count. For large individuals of *M. rigbyae* the second count was relatively low, resulting in younger age estimates. The resulting coefficients of variation (CV) and average percent errors (APE) between the two counts were less than 5%. The error percentage obtained was considered acceptable (typically < 10%,

Campana 2001) and it can be assumed that the reader counted the growth increments with good precision. The mean value between two counts was considered for age estimates. For the additional 37 beaks the counts could be performed only once.

2.4.4 Age estimates and reproductive biology

2.4.4.1 *Megaleledone setebos*

The size distribution of the 48 *M. setebos* from 2012 was biased towards small specimens (DML $\bar{x}$ ♂ 75.3; ♀ 58.1 mm) in the maturity stage II. Most animals weighed less than 500 g, which skewed the results. Beaks of three male specimens (BM 6,000 g, 1,598 g and 3,858 g) that were captured in 1989, 2014, and 2016 were also examined resulting in 51 individuals with increments counted.

Assuming daily deposition of growth increments, age estimates ranged from 98 to 906 days (d). Most males were immature (< stage IV, no spermatophores) and females had small developing oocytes (Table 3). Immature specimens had 98 to 298 increments (BM 10-190 g, $\bar{x}$ = 64 g). In the smallest female (stage II, DML 27 mm, 153 increments), the ovary contained many spherical oocytes (diameter ~ 1mm). Specimens in maturity stage III consisted of one male BM 682 g and two females BM 519 and 670 g, which were estimated 546, 509 and 526 d old, respectively. In four submature males (BM 1882 - 5190 g) between 671 and 792 increments were counted. Two immature females (stage III, 1326 g and 2860 g) had 678 and 719 increments. The beaks of the largest submature male (BM 9760 g, stage IV) captured during the 2012 cruise were not available for age estimation.

Table 3. Summary of reproductive attributes examined from females and males at advanced maturity of different species. DML - dorsal mantle length (mm); BM - body mass (g); GSI - gonadosomatic index; Oocyte N.g^{-1} - number of oocytes per gram of BM; Sperm.length - spermatophores length in mm; Sperm. (N) - number of spermatophores per individual; N - specimens examined. Mean values given in parentheses.

Species (females)	DML range (mm)	BM (g)	GSI (%)	Number of Oocytes	Oocyte Length (mm)	Oocyte N.g^{-1}	Oocytes measured N	N ind
A. polymorpha	55-83 (72)	52 - 116 (88)	2 - 13 (7)	38 - 62 (50)	8 - 14 (9)	0,2 - 0,6 (0,4)	100	2
*M. setebos**	46 - 68 (61)	43 - 184 (101)	< 0.5	150-340 (241)	1 - 2 (1.5)	1,4 - 5,9 (2,8)	350	7
M. rigbyae	65-86 (74)	112 - 256 (167)	1 - 5 (3)	59 - 80 (70)	5 - 15 (12)	0,3 - 0,5 (0,4)	140	3
P. aequipapillae	46 - 60 (57)	29 - 69 (54)	5 - 14 (8)	14 - 40 (25)	2 - 16 (10)	0,3 - 1,1 (0,5)	413	17
P. charcoti	40 - 77 (58)	28 - 124 (65)	3 - 18 (9)	30 - 94 (55)	1 - 18 (12)	0,6 - 2 (1)	302	15
P. felix	52 - 70 (64)	61 - 99 (73)	3 - 15 (8)	21 - 22 (21)	5 - 15 (10)	0,3 - 0,3 (0,3)	43	2
P. turqueti	66	78	12	35 - 35 (35)	3 - 18 (12)	0,4	35	1

Species (males)	DML range (mm)	BM (g)	GSI (%)	Sperm. length range (SpL mm)	Sperm. (N)	N ind
A. polymorpha	50 - 74 (72)	73 - 75 (74)	5 - 5 (5)	28 - 74 (64)	1 - 8 (4)	3
M. rigbyae	69 - 84 (76)	110 - 184 (147)	9-16 (11)	41 - 144 (69)	5 - 26 (16)	11
P. aequipapillae	36 - 78 (48)	15 - 66 (35)	5 - 11 (8)	22 - 119 (64)	1 - 8 (3)	78
P. charcoti	43 - 57 (49)	22 - 40 (30)	6 - 10 (8)	23 - 70 (47)	1 - 9 (4)	19
P. felix	45 - 67 (54)	26 - 136 (89)	7 - 9 (8)	36 - 72 (53)	1 - 6 (3)	16
P. turqueti	95	336	4	52 - 92 (72)	2	1

* Only immature females of *Megaleledone setebos* were observed (stages II and III).

Two male specimens collected in 2014 and 2016 (stage III, DML 172 mm, 1598 g; stage IV, 205 mm, 3858 g) had 628 and 811 increments respectively. The larger specimen had one developed spermatophore (SpL 169 mm) and two more developing. The largest beak examined belonged to a large specimen (sex undetermined; DML 210 mm; ~ 6,000 g) captured in the Weddell Sea in 1989 and it had 906 ± 8 increments. Fig 8 shows a box-plot of the range of the number of growth increments at each maturity stage.

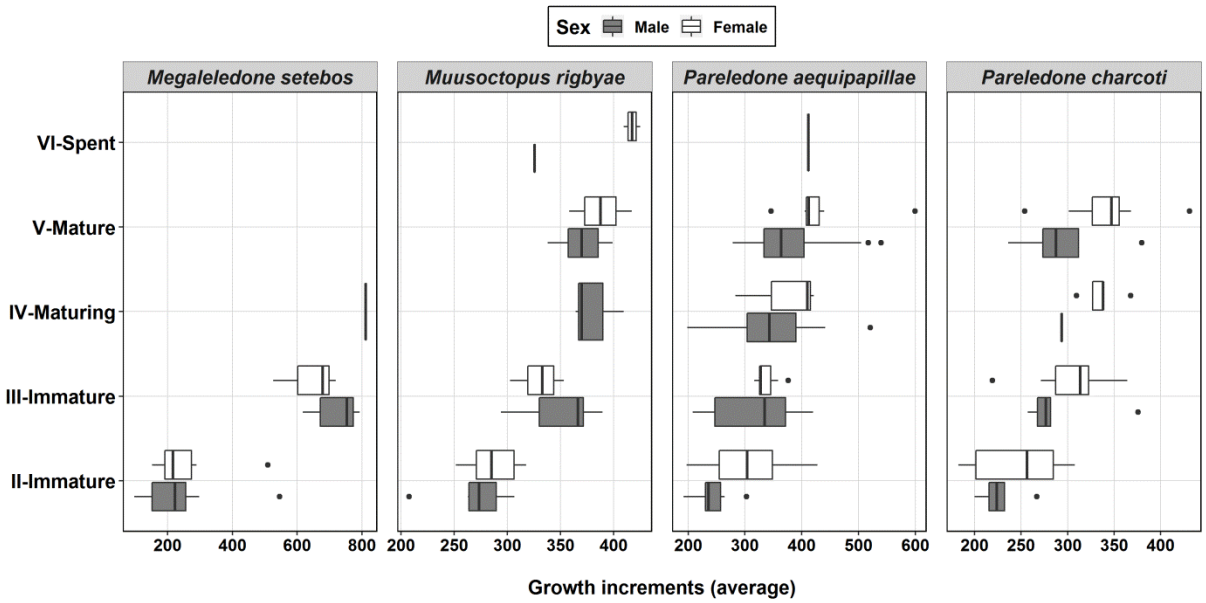

Fig 8. Number of growth increments (mean from two counts) at different maturity stages for the four main species analyzed. Vertical bars indicate presence of only 1 specimen. The largest specimen of *Megaleledone setebos* is not included.

2.4.4.2 *Muusoctopus rigbyae*

Muusoctopus rigbyae exhibited a broad range of ages and maturity stages (Fig 8). We examined 59 specimens from 2012 and increments were quantified for 38 (23 ♂, 15 ♀). Increment number in males (DML 46-84 mm, BM 40-184 g) ranged from 207 to 409. Females (DML 31-86 mm, BM 24-256 g) had between 251 and 425 increments. Mature males (> stage IV) had between 325 and 409 increments ($\bar{x}$ = 369), while mature females had between 358 and 425 increments ($\bar{x}$ = 402).

The ovaries of mature females contained between 59 and 80 oocytes (oocyte length 6 to 15 mm) (Table 3; Fig 9). The two oldest females showed signs of the onset of spawning. One partially spent female (DML 65, BM 112 g, 409 ± 17 increments) had a few fully developed oocytes and some atretic oocytes attached to stalks inside the gonads. The oldest female (DML 69.6, BM 136 g, 425 ± 5 increments) was completely spent with 70 empty follicular folds inside the ovary. There was no evidence of mating (e.g. spermatangia) in either of these females. The males in the most advanced state of maturity (stage V) had 5 to 26 ($\bar{x}$ = 16) fully developed spermatophores (SpL 40-144 mm; $\bar{x}$ = 72 mm). The testis and the spermatophoric complex of one spent male (DML 50, BM 97, 325 ± 24 d) were

completely empty. Most of the specimens in advanced maturity had more than 350 growth increments (Fig 8).

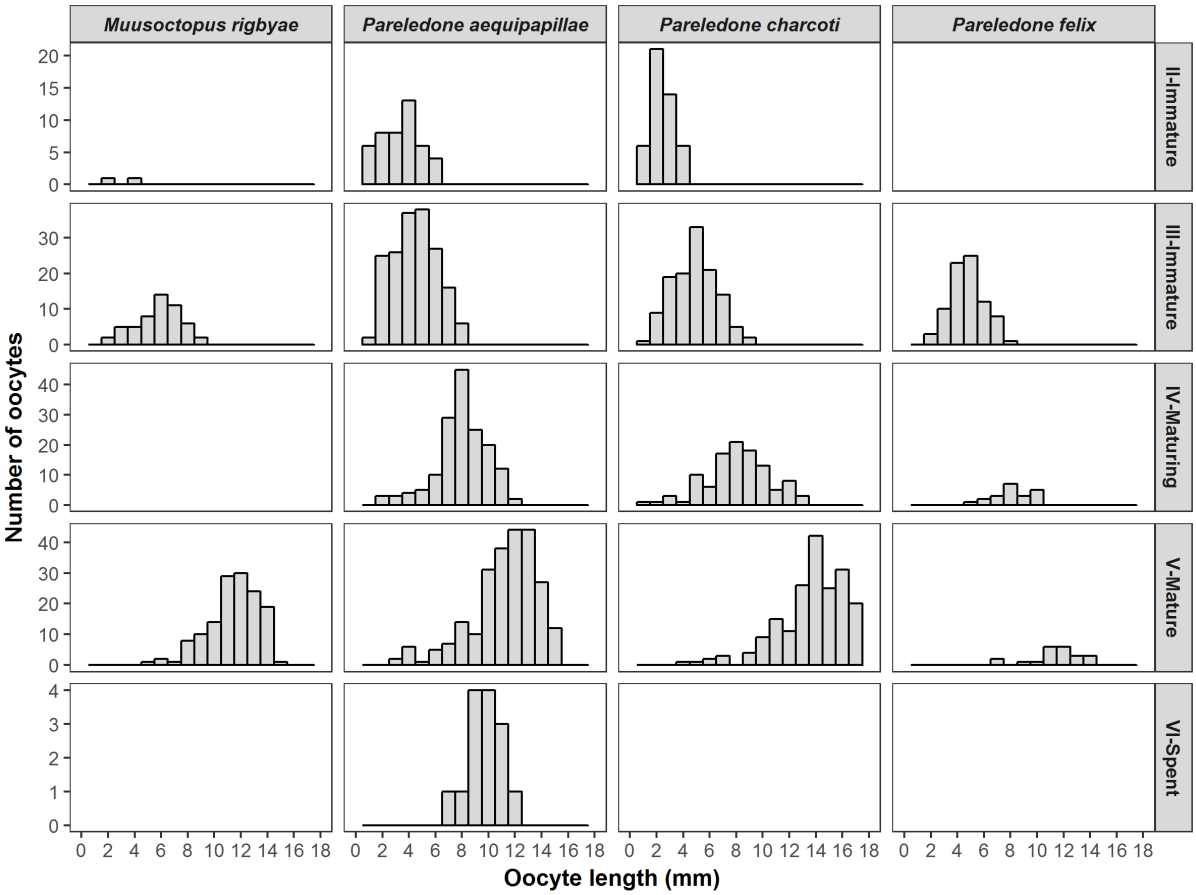

Fig 9. Length frequency distribution of oocytes at different maturity stages for *Muusoctopus rigbyae*, *Pareledone aequipapillae*, *Pareledone charcoti* and *Pareledone felix*.

2.4.4.3 *Pareledone aequipapillae*

We examined 212 *Pareledone aequipapillae* (ranging from juveniles to mature) and increments were quantified in 133 of them (82♂, 51 ♀). Males (DML 24-78 mm, BM 4-90 g) had between 192 and 540 increments. Females (DML 16-60 mm, BM 2-69 g) had between 197 and 599 increments. Mature males had between 272 and 540 increments ($\overline{x}$ = 365) while mature females had between 346 and 599 increments ($\overline{x}$ = 433) (Fig 8).

Males had up to eight developed spermatophores (SpL 40-140 mm, $\overline{x}$ = 62 mm) inside the Needham's sac. Mature females had usually less than 40 oocytes (oocyte length ~ 10 mm, Table 3). A spawning/spent female (DML 57, BM 50 g, 411

± 38 increments) had 14 follicular yolk oocytes attached to stalks with lengths from 8 to 13 mm ($\bar{x}$ = 10 mm) and 20 empty follicular folds.

2.4.4.4 *Pareledone charcoti*

The size structure as well as reproductive biology of *P*. *charcoti* was examined in 176 specimens, and the increments in the beaks were counted in 33 of these. Males (DML 28-57 mm, BM 5-40 g) had between 200 and 379 increments, and females (DML 25-77 mm, BM 6-125 g) had between 182 and 431. Mature males (average DML 48 mm and BM 32 g) had between 236 and 379 increments ($\bar{x}$ = 296). Females mature at larger sizes (average DML 60 mm and BM 70 g) and their beaks had between 309 to 431 growth increments ($\bar{x}$ = 355).

One of the largest mature females (DML 77 mm, BM 112 g, 351 ± 1 increments) had a GSI of 18 %, and 65 well-developed oocytes (oocyte length 14-18 mm). Female potential fecundity in *P*. *charcoti* is higher than *P*. *aequipapillae* (Table 3). The number of the spermatophores is similar in both species (up to 9, $\bar{x}$ = 4), but they are on average smaller in *P*. *charcoti* (SpL 23-70 mm, $\bar{x}$ = 47).

The beaks of the 11 females that were kept alive at AWI had 219 to 364 growth increments. In spite of the fact that no specific information about the initial size of these animals was provided, all the specimens that were kept in aquaria were measured during the cruise and none of which were hatchlings (N= 250; DML 32-65, $\bar{x}$ = 42 mm; BM 7-119, $\bar{x}$ = 44 g). Six of the AWI specimens were still immature (stage III; DML 35-42 mm, BM 22-33 g), had between 219 and 342 growth increments and their ovaries had between 57 and 75 developing oocytes (< 6.5 mm). The other five females were submature and early mature (DML40-46, BM 28-46) and had between 301 and 364 growth increments in their beak LWS. Their ovaries contained 30 to 62 oocytes (7-15 mm). One female had 49 atretic oocytes (< 5 mm) and 2 large follicular oocytes (length = 10 mm).

2.4.5 Increment counts in other octopod species

2.4.5.1 *Adelieledone polymorpha*

The beaks of 13 *A*. *polymorpha* (6 ♂ and 7 ♀) were examined. All females were juveniles (stage II) and increment number ranged from 223 to 304. Submature males (stage IV, DML 50-74mm, BM 62-88 g) had between 218 and 510 growth increments. Their spermatophoric complex contained up to 8 developing spermatophores (SpL 28-74 mm; $\bar{x}$ = 64 mm). Mature females ranged from 55 to 83

mm DML ($\bar{x}$ = 72 mm) with a GSI up to 13%, but age was not estimated. Their ovaries contained 38 to 62 vitellogenic oocytes (diameter = 8-14 mm), but ripe eggs were absent. One spent female (DML 77 mm, BM 52 g) had a flaccid and weakened muscular body and its oviducal glands were pale grey in color, strongly suggesting a senescent state. Inside its ovary, 130 undeveloped oocytes (~ 2.5 mm) were present and connected to epithelial stalks. No vitellogenic oocytes, ripe eggs or post-ovulatory follicles were observed.

2.4.5.2 *Pareledone aurata (Allcock, 2005)*

Two male *P*. *aurata* (stage IV) were examined. The upper beak of the smaller one (DML 40 mm BM 29 g) had between 367 and 470 increments ($\bar{x}$ = 421 ± 54). The larger specimen (DML 43 mm, BM 37 g) had between 371 and 420 increments ($\bar{x}$ = 395 ± 24). However, both individuals had beaks that produced inaccurate counts with a CV higher than 10% and were not suitable for age estimates.

2.4.5.3 *Pareledone felix (Allcock, Strugnell, Prodohl, Piatkowski and Vecchione, 2007)*

Growth increments were quantified in 15 specimens of *P*. *felix* (8 ♂; 7 ♀). Males (DML 22-54 mm, BM 4-44 g) had between 214 and 331 increments. Females (DML 20-52 mm, BM 2-62 g) had between 198 and 301 increments.

2.4.5.4 *Pareledone turqueti*

Pareledone turqueti is the largest species of the genus, the beaks of seven males (DML 38-100 mm; BM 29-613 g) were examined and exhibited between 245 and 656 increments. Three submature males at stages III and IV (BM 24-35 g) had between 245 - 328 increments. The four mature males (BM 267 - 613 g) had 458 - 656 increments. All four females were immature (stages II-III; DML 32-59, BM 9-113 g) and had 246 - 459 increments.

2.4.6 Growth

Growth curves were adjusted using the average number of growth increments (g*inc*) in upper beaks LWS and body mass for *M*. *setebos*, *M*. rigbyae, *P*. *aequipapillae* and *P*. *charcoti* (Fig 10). All curves were adjusted for sexes combined (appendix Supplement S2, Table 2). In *M*. *setebos*, the number of growth increments correlates well with the increase in body mass (R^2 = 0.94). For other species, the

correlation is significant but the power curve provided modest fits to the plot (R^2 = 0.4 - 0.6).

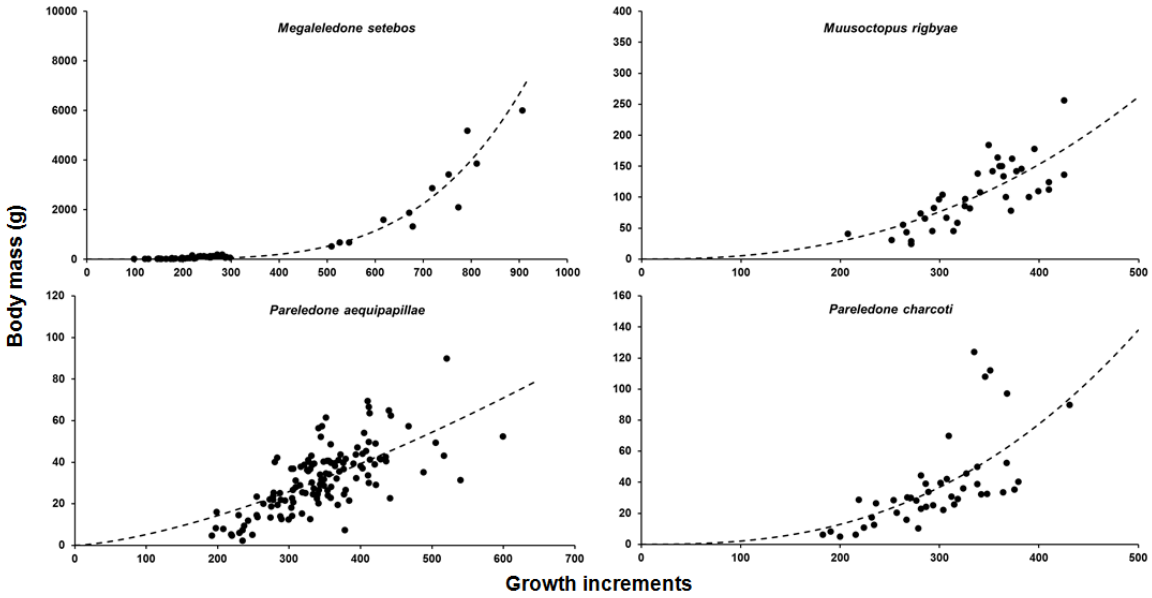

Fig 10. Growth curves showing the relationship between growth increments number (N) and body mass (g) of four species analyzed in this study. Clockwise species order: *M*. *rigbyae*, *P*. *charcoti*, *P*. *aequipapillae* and *M. setebos*.

Assuming a daily periodicity of the growth increments, the giant Antarctic octopus *M*. *setebos* exhibited the highest growth rates (*G*), with values between 0.13 and 2.5% BM d^{-1} (Table 4). *Muusoctopus rigbyae* and *P. charcoti* have intermediary *G* values (0.03 to 2.23% BM d^{-1}), and *P*. *aequipapillae* had the lowest (0.02 to 1.6% BM d^{-1}).

Table 4. Instantaneous growth rates (*G*) for four Antarctic octopod species. Avr BM – Average body mass (g) for the age class. Dash indicates data unavailability.

Megaleledone setebos						
		♂			♀	
Age class	N	Avr BM (g)	*G*	N	Avr BM (g)	*G*
90 - 150	5	14.93	-			-
151 - 210	5	20.30	0.51	9	29.82	-
211 - 270	8	93.92	2.55	5	101.52	2.04
271 - 330	2	128	0.52	5	115.16	0.21
331 - 390	-	-	-	-	-	-
391 - 450	-	-	-	-	-	-
451 - 510	-	-	-	-	-	-
511 - 570	1	682	0.70	2	594	0.68
571 - 630	1	1598	1.42	-	-	-
631 - 690	1	1882	0.27	1	1326	0.67
691 - 750	-	-	-	1	2860	1.28
751 - 810	3	3568	0.53	-	-	-
811 - 870	1	3858	0.13	-	-	-
Muusoctopus rigbyae						
		♂			♀	
Age class	N	Avr BM (g)	*G*	N	Avr BM (g)	*G*
90 - 150	-	-	-	-	-	-
151 - 210	1	40.00	-	-	-	-
211 - 270	2	49.23	0.35	1	31.00	-
271 - 330	6	74.67	0.69	8	63.63	1.20
331 - 390	11	134.93	0.99	3	138	1.29
391 - 450	3	137.33	0.03	3	168	0.33
Pareledone aequipapillae						
		♂			♀	
Age class	N	Avr BM (g)	*G*	N	Avr BM (g)	*G*
90 - 150	-	-	- -	-	-	-
151 - 210	2	6.30	- -	1	8.40	-
211 - 270	4	12.80	1.18	8	10.25	0.33
271 - 330	21	25.11	1.12	15	27.78	1.66
331 - 390	31	34.80	0.54	17	32.65	0.27
391 - 450	16	40.14	0.24	9	54.26	0.85
451 - 510	3	42.27	0.09	-	-	-
511 - 570	3	54.85	0.43	-	-	-
571 - 630	-	-	-	1	56.00	0.02
Pareledone charcoti						
		♂			♀	
Age class	N	Avr BM (g)	*G*	N	Avr BM (g)	*G*
90 - 150	-	-	-	-	-	-
151 - 210	1	5.10	-	2	7.31	-
211 - 270	8	19.44	2.23	3	23.28	1.93
271 - 330	4	30.23	0.74	13	34.60	0.66
331 - 390	2	37.86	0.38	10	68.10	1.13
391 - 450	-	-	-	1	89.73	0.46

2.5 Discussion

2.5.1 Distribution

The occurrence of several *Pareledone* species in the study region corresponds with previous reports that suggested that the South Shetland Islands are a hotspot for speciation of the octopod genus *Pareledone* (Allcock *et al.* 2011). Overall, the majority of species were captured between 250 and 350 m bottom depths. The dominance of *Pareledone charcoti* in depths of less than 200 m agrees with Allcock (2005), who attributed previous deeper records of this species to potential misidentifications of other *Pareledone* species (Kuehl 1988). The high abundance of *P*. *charcoti* in shallow waters might be related to a prey preference for amphipods (Piatkowski *et al.* 2003). Amphipods are known to dominate the mobile benthic fauna at ~25 m (Jażdżewski *et al.* 1991, Strugnell *et al.* 2017). In the shelf and slope areas of the Weddell Sea, *Pareledone* species including *P*. *charcoti* occur from 200 to 800 m depths, similar to the distribution of amphipod species (Allcock *et al.* 2001, De Broyer *et al.* 2004). However, studies on the feeding ecology of Antarctic octopods are scarce (Daly 1996, Piatkowski *et al.* 2003).

Pareledone turqueti and *A*. *polymorpha* specimens were collected at a broad range of depths (100 - 475 m). Both species have circumpolar distribution and occur in depths between 100 m and more than 1000 m (Strugnell *et al.* 2012, 2017). Specimens of *Muusoctopus rigbyae* were collected in various depth strata. They were most abundant around 300 m at the Antarctic Peninsula, which is shallower than the typical depths in which this genus occurs in other ocean regions (Vecchione *et al*. 2009). This characteristic may be related to the evolutionary history of this group, where deep-sea *Muusoctopus* that originated from the northern hemisphere colonized the Southern Ocean five million years ago (Vecchione *et al.* 2009, Strugnell *et al.* 2011). While specimens of *Megaleledone setebos* are typically found deeper than 250 m, there are shallower records. The type specimen of *M. setebos* was collected in a tide pool on Ross Island in 1911 (Robson 1932). Further, a large specimen (~ 8 kg) was captured by a SCUBA diver at 20 m in the Davis Sea in 1966 (Nesis & Propp 2003). This broad depth distribution highlights the lack of environmental stratification around the South Shetland Islands shelf and slope, and the eurybathic nature of the studied species. The understanding of the depth

distribution of Southern Ocean Octopodidae remains biased as a result of the selected depth strata for trawling.

2.5.2 Size distribution and maturity

In all examined species, except for *M*. *rigbyae*, maturation advanced gradually with size for both sexes. Vecchione et al. (2009) reported several maturity stages over a narrow size range for *M*. *rigbyae* females and suggested that they may mature rapidly after reaching a threshold size. Female biased sexual size dimorphism was observed in *P. charcoti*. Among the papillated *Pareledone* species which are typically of small size (BM < 200 g), *P*. *charcoti* females are mature at ~130 g. Specimens of *P*. *turqueti* (BM > 500 g) and *M*. *setebos* (BM > 10,000 g) can attain moderate to large body dimensions, while mature *M*. *rigbyae* and *Adelieledone* spp. are of intermediate size (BM < 500 g).

Size distribution of species described here corresponds with that reported by Barratt (2009), who investigated the same group of species from the Antarctic Peninsula (< 1000 m). The animals assigned by Barratt (2009) as *Benthoctopus* cf. *levis* are likely *M*. *rigbyae* (Vecchione *et al.* 2009), since the specimens were captured in the same region of those from our study. The relationship between body mass and dorsal mantle length for seven of the study species correspond with Piatkowski et al. (2003). For *P*. *charcoti* males and both sexes of *M*. *rigbyae*, the length weight curves did not produce a strong fit (r^2 < 0.7). This may be due to the small sample size and the inclusion of spent individuals (appendix Supplement S2, Table 1). Body weights can decrease dramatically after the release of gametes biasing the correlation between mantle length and weight.

The observed co-occurrence of a broad range of sizes and maturity stages suggests that reproduction and hatching may occur throughout the year. Previous publications already propose a lack of strong seasonal reproductive activity in Southern Ocean incirrate octopods (Kuehl 1988, Yau *et al.* 2002, Barratt *et al.* 2008). Southern Ocean octopods inhabiting waters of -1.8 to 2°C produce relatively large eggs (10-40 mm) which require a longer embryonic development time than species in temperate waters, and likely result in well-developed benthic hatchlings (Boletzky 1994, Robison *et al.* 2014). These hatchlings are miniature adults capable of feeding directly on benthic fauna and therefore independent from seasonal plankton blooms (Collins *et al.* 2006). However, most of the Southern Ocean expeditions are restricted to the austral summer (November-April), and sampling efforts throughout the year are

necessary in order to elucidate whether or not octopod reproductive activity is coupled with Antarctic seasons.

Similar to Yau et al. (2002), who investigated *P. turqueti* and *A*. *polymorpha* around South Georgia, we did not find mated, spawning, or brooding females. Their absence could be the result of the species having a nesting behavior in areas that are unsuitable for trawling (*e.g*. sponge and rock bottoms). Aggregations of brooding females were observed for *Muusoctopus* and *Graneledone* species in the North Pacific (Voight & Grehan 2000) and on a rocky outcrop off Costa Rica in the Central Pacific Ocean (Hartwell *et al.* 2018).

2.5.3 Beak morphology and growth increments

The diversity of beak morphologies observed in Southern Ocean octopods suggests that the species may occupy different ecological niches. The species examined had the typical Octopodidae beak shapes and may potentially feed on similar prey (Daly 1996, Piatkowski *et al.* 2003, Allcock 2005, Vecchione *et al.* 2009). The beaks of *M*. *setebos* show the most robust structure. The beaks of *Adelieledone* spp. have an unusual morphology, and lack a distinct rostral tip on the upper beak but they do have a delicate inverted rostrum on the lower beak. Daly and Rodhouse (Daly *et al.* 1994) argued that differences in beak morphology between *A*. *polymorpha* and *P*. *turqueti* may indicate differences in foraging and diet. Diet studies showed that both these species prey on crustaceans and polychaetes, but that *P. turqueti* in addition feeds on shelled bivalves and gastropods which requires a more robust beak (Daly 1996).

The increment widths (W_{inc}) in the beak lateral wall surfaces (LWS) of the examined species were on average narrower than previously reported for other octopod species. The maximum W_{inc} of ~78 µm in *M*. *setebos* is close to the lower limit of the W_{inc} range observed in the beaks of the temperate *Octopus vulgaris* Cuvier, 1797 (75-100 µm; Perales-Raya *et al*. 2010). The W_{inc} of 30-50 µm reported for the tropical *Octopus maya* Voss and Solís, 1966 (Rodríguez-Domínguez *et al.* 2013) are similar to those from *M*. *setebos*, but almost two times wider than the average widths observed in other examined Antarctic species (W_{inc} 19-25 µm). Although growth increments are narrow in Antarctic species, the percentage of agreement between the two age estimates was considered acceptable to this type of analysis, with an average error of less than 7% (Campana 2001). The error rates

between two increment counts presented here are below those reported by other authors (4-10%) who used similar methods (Perales-Raya *et al.* 2010, Rodríguez-Domínguez *et al.* 2013).

2.5.4 Age estimates

To date, few studies have investigated age and lifespan in cold water octopods (O'Dor & Macalaster 1983, Wood 2000, Barratt & Allcock 2010, Robison *et al.* 2014). We applied for the first time the quantification of growth increments in upper beak lateral wall surfaces to estimate age of Antarctic incirrate octopods. Assuming a daily periodicity of increment formation, most of our species reach maturity during the second year of their life. Large specimens of *M*. *setebos* were still immature in their third year of life, and they may have a lifespan of several years. Barratt and Allcock (2010) used the quantification of growth increments in the stylet microstructure of the Antarctic *M*. *setebos* and the deep-sea octopus *Bathypolypus sponsalis* to infer lifespans. While the periodicity of stylet increment formation is not validated for these species, their results suggested that the lifespan of *M*. *setebos* is between 3 to 4 years and less than 1 year in *B. sponsalis*. The stylet of their largest *M*. *setebos* specimen had 1,077 growth increments (DML 190 mm, BM ~7,000 g) (Barratt & Allcock 2010). This estimate is close to the 906 growth increments observed in the beaks of our largest *M*. *setebos* (DML 210 mm, BM ~6,000 g).

For species of intermediate size, the oldest age was inferred to *P*. *turqueti*, with the upper beaks of the most mature male (DML 100, BM 613 g) containing 656 increments. Specimens of *M*. *rigbyae* also seem to reach advanced maturity during their second year, and the oldest female had 425 growth increments. Among the small sized octopods, *P*. *aequipapillae* appears to have the longest lifespan with the most mature animals being older than 500 days. Specimens of *P*. *aequipapillae* and *P*. *charcoti* exhibited a broad range of both size and age for mature animals, which suggests that the final stages of the reproductive activity may take a considerable portion of their lifespan.

Captured immature individuals of *P*. *charcoti* that were kept in aquaria under natural temperature regime (0°C) for more than 12 months had between 219 to 364 beak growth-increments. The beaks of these individuals exhibited the same growth-increment pattern observed in other specimens collected from nature during the expedition, and there were no checks or stress marks in the beak lateral wall surfaces. Although the periodicity of beak-increment formation remains to be properly

validated for our study species, the fact that the number of beak increments is lower than the number of days that the animals were kept in captivity strongly suggests that increments in *Pareledone* need more than one day to be formed.

The reduced W_{inc} of Antarctic species could be related to the relatively slow development of increments under low temperatures. Canali et al. (2011) investigated age of *O. vulgaris* from the Bay of Naples and observed that recent growth increments in the posterior region of beak lateral wall surfaces were narrower in animals captured during winter months than those captured in summer. The authors argued that the rate of increment formation must be governed by a biological rhythm that depends on metabolic activity, which is mainly influenced by temperature. Perales-Raya et al. (2018) developed a model for estimating age of wild *O*. *vulgaris* paralarvae using growth increments from beaks of animals reared at different temperatures. They observed that growth increments were formed daily in animals reared under optimal temperature conditions of 21°C, while in individuals that were kept at ~14°C, one increment was formed every 1.6 days. Their model predicts that at 10°C, a growth increment would be formed every 7th day. Following this model the growth increments in the beaks of Antarctic octopods living at 0°C would very likely require more than one day to be formed, as also suggested by the preliminary validation experiments described here.

Further indirect evidence also supports the hypothesis that growth increments require several days to be formed at low temperatures of the Antarctic Ocean. The instantaneous growth rates (*G*) estimated here of 0.38 to 2.23% BM d^{-1} for *P*. *charcoti* (under the assumption of daily increments) were higher than those observed in animals kept in laboratory (0.08 to 0.13% BM d^{-1}) (Daly & Peck 2000). If the periodicity of beak growth-increment formation is assumed to be 7 days in *P*. *charcoti*, the mature animals examined here would be between four and eight years old. This assumption reduces the estimated *G* to values of 0.05% to 0.32% BM d^{-1}, which are closer to the growth rates estimated under lab conditions (Daly & Peck 2000). A lifespan of about eight years for mature *P*. *charcoti* is congruent with the lifespan estimates derived from life-history traits of octopods living at temperatures below 5°C and observed in situ (Robison *et al.* 2014, Schwarz *et al.* 2018). In this context it is worth noting that there is an anecdotal record of a *P*. *charcoti* specimen that was kept alive for about eight years at the Alfred Wegener Institute (Dr. Felix Mark, *pers. comm.*).

In comparison, the quantification of growth increments in stylets of the temperate species *Eledone cirrhosa* (Lamarck, 1798) from the north Atlantic (living at 11-14°C), suggests a maximum lifespan of 516 days with an *G* values of 0.97 to 1.15% BM d^{-1} (Regueira *et al.* 2015). Mangold and Boletzky (1973) reported a growth rate of 0.9% BM d^{-1} for *O. vulgaris* at its lower temperature limit of 10°C. This value is similar to the 0.8% BM d^{-1} found for *Bathypolypus arcticus* (Prosch, 1849) reared at its upper temperature limit of 10°C (Wood 2000). Wood (2000) also investigated growth in laboratory-reared *B*. *arcticus* at 6 °C and estimated that if juveniles continued to grow at the same rate of 0.38% BM d^{-1}, they would reach their maturity size of 70 g after 6 years. This was a conservative estimate since animals of this species usually live in waters of 4 ± 2°C, and at this temperature females may brood their eggs for 400 days. Moreover, respiration rates of *P*. *charcoti* are the lowest ever measured for a benthic octopod species. Daly and Peck (2000) observed that after 40 days of fasting, respiration rates of one specimen dropped from 10.47 to 9.52 mg O_2 $kg^{-1}h^{-1}$. This respiration value is 1/4 of *E*. *cirrhosa* at 11°C, and 1/8 of *O*. *vulgaris* at 21°C (Daly & Peck 2000).

The above mentioned examples suggest that the growth rates calculated in our study might be substantially overestimated due to the assumption of daily increment formation. Such overestimation is further supported when considering the physiology behind the formation of increments. The time required to complete feeding related processes (e.g. food consumption and digestion) in Antarctic marine invertebrates is two to five times longer than in temperate relatives (Peck 1998). Embryogenesis and larval development in Antarctic marine ectotherms are also considerably slower than in temperate species (Peck 2002, Peck *et al.* 2006). Beak growth results from the metabolic and secretory activity of cells distributed in the buccal mass (Dilly & Nixon 1976, Houlihan *et al.* 1990). If all metabolic processes in the organism are delayed under the low temperatures of the Antarctic environment, there is no reason to believe that the process of growth-increment deposition happens at a rate similar to that of species living in temperate waters.

It is also worth noting that because hatchlings are miniatures of the adults, with well-formed beaks, some growth increments may be deposited during the embryonic development. The distinct increments in the anterior rostrum region are potentially formed during the embryonic phase and may result in an overestimation of the adult lifespan. Validation experiments are the next step to determine increment

formation in cold water octopod beaks. The administration of oxytetracycline and the injection of calcofluor in husbandry experiments, are successful age validation methods in stylets and beaks respectively (Hermosilla *et al.* 2010, Bárcenas *et al.* 2014, Perales-Raya *et al.* 2014) and should also be suitable for experiments on Antarctic octopods which have been successfully kept under laboratory conditions (Daly & Peck 2000, Oellermann 2015).

Assuming a daily periodicity for the growth increments in the beaks of Antarctic octopods, females mature during their second year and would require up to 4 years for brooding the eggs at 0°C (Robison *et al.* 2014, Schwarz *et al.* 2018). This assumption would result in a lifespan of about 6 years for the females of intermediate sized species, and more than 7 years for the large *M*. *setebos*. The alternative scenario, where one growth increment represents up to 7 days (i.e. weekly laid), would result in a lifespan of 8-12 years for *M*. *rigbyae* and *Pareledone* spp., and more than 15 years for *M*. *setebos*. The relatively high longevities of Antarctic octopods fit the increased lifespans observed for other marine invertebrates inhabiting Antarctic waters (Vogt 2012, Moss *et al.* 2016).

Thorson's rule (Mileikovsky 1971), a hypothesis that states that there is a positive correlation between egg sizes, embryonic duration, latitude and depth in benthic ectotherms, has been tested and validated for several taxa including octopods (Ibáñez *et al.* 2018). Due to the strong seasonality of plankton production in the Southern Ocean, it may be evolutionary advantageous to produce relatively few but well-developed benthic offspring instead of large numbers of pelagic planktotrophic larvae. Life history theory predicts that the allocation of energy to the production of fewer but larger offspring with extended embryogenesis (or parental care), slow growth rates, and late maturity is found mainly in species with longer lifespans (Stearns 1992, 2000). The life history traits of Antarctic octopods resemble those from animals displaying a slower pace of life and longer lifespans.

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Authors' contributions

R.S., H.J.H. and U.P. designed the study; C.N. collected octopods during the Polarstern 2012 expedition to the Southern Ocean; R.S. processed the specimens, collected, prepared and processed the beaks for age estimation, extracted and analyzed reproductive systems and wrote the first manuscript draft; All authors wrote and edited the manuscript for journal submission.

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Chapter 3

Quantification of beak increments to study the pace of life in pelagic deep-sea Octopodiformes *Japetella diaphana* and *Vampyroteuthis infernalis*

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3.1 Abstract

The pelagic octopodiforms *Japetella diaphana* and *Vampyroteuthis infernalis* are common and charismatic components of the pelagic deep-sea ecosystem occurring in all oceans worldwide. The life history of these cephalopods remains very poorly known. Their habitat temperatures, metabolic rates, feeding and reproductive strategies all together suggest that the pace of life in these species is slower than in neritic octopod species, but age estimations are absent to date. The deposition of growth increments in hard body parts (e.g., beaks, stylets) has been validated to be daily in temperate and tropical octopods, but the periodicity of increment deposition remains unknown for most of the deep-sea and high latitude counterparts. This study investigated size at maturity, reproductive strategy and the number of growth-increments in the upper beak lateral walls (LWS) of *J. diaphana* and *V. infernalis*. We used a unique collection of specimens ranging from early juveniles to adults for both species. Mature animals of *J. diaphana* had mantle length (ML) ranging from 53-144 mm and body mass (BM) ranging from 18-235 g, while the size range of mature specimens of *V. infernalis* was ML 66-122 and BM 34-286 g. The number of growth increment in the beaks ranged from 21 to 207 in *J. diaphana* (ML 4.5-144 mm; BM 0.0123-235 g) and from 89-375 in *V. infernalis* (ML 12-136, BM 0.08-286). If daily deposition of increments is assumed, the resulting lifespans are about one to two years for the examined cephalopods. These figures are incongruent with the low metabolic rates and reproductive strategies of the two species, which rather suggest that growth increments may require more than one day to be formed. A specimen of *J. diaphana* captured at 1352 m in the Gulf of California was brooding eggs and had

192-202 increments in its beak. The water at this depth is 3-4°C, and at this temperature embryos may require as much as two years to develop. Our results suggest a non-daily rate of increment-deposition in beaks of *J. diaphana* and *V. infernalis* and illustrate the urgency for validation studies in deep-water invertebrates, to accurately determine age and longevity. This is necessary to better understand life histories of invertebrates in the largest but least studied habitat on the planet.

3.2 Introduction

The pelagic deep sea is the Earth's largest habitat and it holds an enormous biodiversity of which most remains to be discovered (Robison 2009). In open ocean regions, sunlight supports primary productivity via photosynthesis in the upper 200 m, the epipelagic zone (Mann & Lazier 2005). Below this depth, the last vestiges of light gradually fade into the mesopelagic zone (200 to 1000 m) or 'twilight zone' until the start of the eternally dark and cold bathypelagic zone which extends from 1000-3000 m (Angel 2003). The meso- and bathypelagic zones comprise the largest marine ecosystems in terms of volume, and organisms inhabiting these environments evolved a variety of life strategies to cope with the cold, dark and high pressure deep-sea pelagic environment (Robison 2004). Bioluminescence is one of the most important ecological traits in the dark deep sea, and most cnidarians, cephalopods and fishes living in these zones can produce light (Martini & Haddock 2017). Life history information on age, growth and reproduction is unknown for most deep living pelagic organisms, in particular invertebrates. However, such data is of importance for understanding oceanic food webs as many pelagic invertebrates are prey for oceanic predators, some of which sustain commercial fisheries (Kubodera *et al.* 2007, Morato *et al.* 2016).

Among the invertebrates inhabiting the meso- and bathypelagic zones, the coleoid cephalopod mollusks (e.g. Decapodiformes: cuttlefish and squids; and Octopodiformes: octopods and the vampire squid) play a pivotal role acting as vectors of energy transfer, from the zooplankton and micronekton to pelagic top predators (Passarella & Hopkins 1991, de la Chesnais *et al.* 2019), and ultimately when cephalopods die after reproduction some species sink to the seafloor where they are consumed by deep-sea bottom scavengers (Hoving *et al.* 2017). Cephalopods can exhibit very different life strategies (Boyle & Rodhouse 2005).

While squids living near the surface or on the continental shelves are fast-growing active swimmers with high energy demand, some deep-sea species adopted a neutrally buoyant more passive lifestyle similar to gelatinous zooplankton (Nesis 2002). Metabolism in pelagic cephalopods, as in all poikilothermic animals, is negatively correlated with temperature and depth of occurrence (Seibel *et al.* 1997, Angilleta 2009). The sub-surface actively swimming squids can have metabolic rates that are 200-fold higher than those of the semi-gelatinous octopodiform species living in mesopelagic oxygen minimum zones (OMZ) at depths of 400-1000 m, differences caused by a combination of both lifestyle and water temperature (Childress & Seibel 1998, Seibel 2007). Examples of pelagic cephalopods that are capable of inhabiting OMZs are the pelagic yellow ring octopus *Japetella diaphana* (Hoyle, 1885) and the vampire squid *Vampyroteuthis infernalis* (Chun, 1903) (Fig 1).

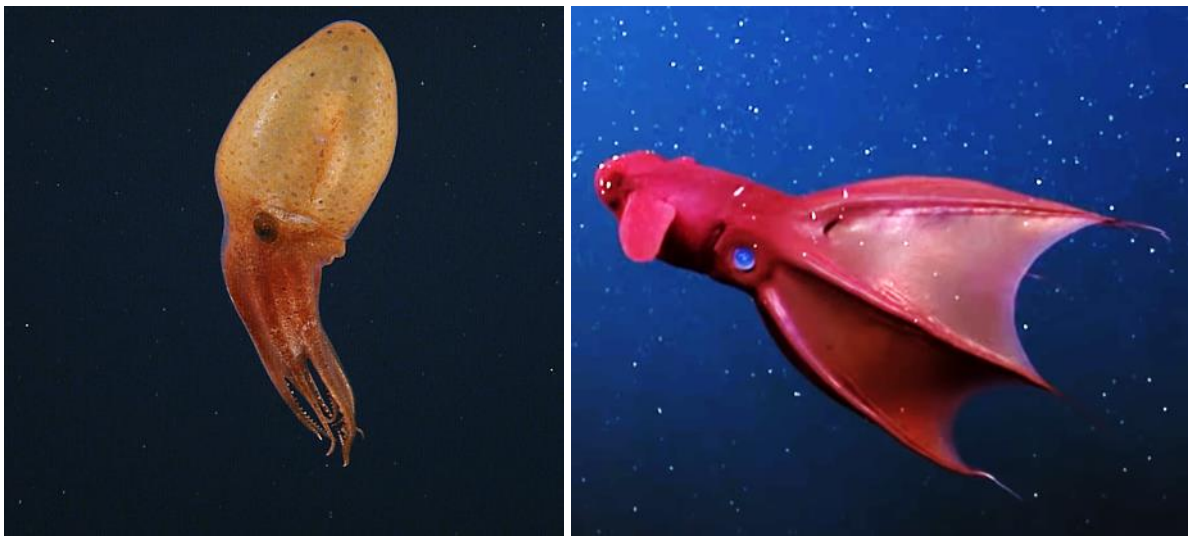

Fig 1. Two octopodiform species that may be encountered inside oxygen minimum zones, *Japetella diaphana* (*left*, © MBARI 2006) and *Vampyroteuthis infernalis* (*right*, © MBARI 2016).

The two species occur in the mesopelagic zone of temperate and tropical oceans worldwide and their vertical distribution may overlap with strong OMZs in certain oceanic regions (Monterey Bay, Gulf of California) (Hoving & Robison 2012, Birk *et al.* 2019). The vampire squid and *J. diaphana* have passive lifestyles, hovering in the water column and potentially using the OMZs as a refuge from predators, which cannot cope with the reduced oxygen levels (Seibel *et al.* 1997). Although aspects such as anatomy and distribution of the two species have been described since the early 1900's (Pickford 1946, 1949, Thore 1949, Chun 1975), basic ecological information on reproduction, feeding habits and trophic position

were until recently not very well known (Hoving *et al.* 2012, 2015a, Gloeckler *et al.* 2018). Direct measurements of their somatic growth rates and the total lifespan are absent.

Age and growth in *J*. *diaphana* were estimated indirectly through mantle length frequency distribution, suggesting approximately two years for this species to reach full maturity (Thore 1949). Vampire squid lifespan is unknown, but its unique reproductive strategy that involves multiple reproductive cycles with intercalate spawning and resting events may require several months per cycle to be completed, and hence suggests a considerably longer lifespan than in neritic octopods which typically live for one year (Hoving *et al.* 2015a). Vampire squid dietary habits rely primarily on suspended particles (*i*.*e*. marine snow) and *J*. *diaphana* is known to feed from a primarily suspended particle-based food web, which seems to supply enough energy to fulfil the low metabolic requirements of the two species (Hoving *et al.* 2012, Gloeckler *et al.* 2018). Estimates of age at maturity based on the habitat temperatures and their life history traits, predict a lifespan of more than three years (Schwarz *et al.* 2018). These indirect lines of evidence, together with the fact that the metabolic rates of these two species are the lowest recorded among deep-sea pelagic cephalopods (Seibel *et al.* 1997), suggest that their pace of life may be slower than in other octopodiforms living in temperate and tropical seas. Our goal is to correlate body size with increments in beaks to determine if increments in hard body structures can provide information on age and longevity in these pelagic octopodiform species.

Age and lifespans of wild cephalopods are typically measured through the interpretation of growth increments in the microstructure of hard parts like statoliths and gladii (squids) or stylets and beaks (octopods) (Arkhipkin *et al.* 2018). The vampire squid statolith consists of a conglomerate of crystals (statoconia) inside the statocyst chambers located in the cartilaginous skull (Stephens & Young 1976, Clarke & Maddock 1988). These structures are very delicate, and fall apart when manipulated (e.g. during extraction) and are not suitable for growth-increment visualization (Hoving, *pers*. *comm.*). Statoliths of *J*. *diaphana* resemble those found in other octopod species and do not have visible increments in their microstructure (Clarke 1978, Arkhipkin *et al.* 2018). Unlike most other octopods *Japetella* does not have stylets. Stylets are the vestigial internal shells, which are used for age estimation in neritic octopods (Reis & Fernandes 2002, Doubleday *et al.* 2006,

Arkhipkin *et al.* 2018). The vampire squid has a soft and delicate internal shell (*i.e*. gladius) that resembles the teuthoid gladius (Bizikov 2004). This chitin-cartilage structure grows in a tile-like pattern, exhibiting lines that grow in different directions and that are difficult to interpret (Bizikov 2004). Unfortunately, trawled vampire squid frequently have damaged gladii and it is hard to extract the intact gladius from preserved specimens (*personal obs*.).

The cephalopod beaks have been used to estimate age and growth in octopods and squids (Hernández-López *et al.* 2001, Perales-Raya *et al.* 2010, Hu *et al.* 2016, Liu *et al.* 2016). In a few temperate and tropical octopod species (living in waters of 17-25 °C), the periodicity of the growth-increment deposition was validated to occur daily (Rodríguez-domínguez *et al.* 2013, Perales-Raya *et al.* 2014). Both *J*. *diaphana* and *V*. *infernalis* have chitinous beaks which exhibit growth-increments that are arranged in a regular pattern (Young 2014). Although the periodicity of increment deposition in the beaks of *J*. *diaphana* and *V*. *infernalis* is unknown, the total number of increments of mature animals has never been investigated. As life-history traits suggest lifespans exceeding three years for both species (Schwarz *et al.* 2018), one would expect to find more than ~ 360 growth increments in beaks of mature specimens, assuming daily rate of increment deposition as found in other octopods. Using a unique collection of specimens, this study will test the hypothesis that growth-increments in beaks of *J*. *diaphana* and *V*. *infernalis* can be used as a tool for direct age estimates. Additionally, we investigate reproductive patterns of *J*. *diaphana*, and discuss how the ecology and life history characteristics of both species fit with the beak increment counts.

3.3 Methods

3.3.1 Collection of specimens

Specimens of *Japetella diaphana* and *Vampyroteuthis infernalis* were obtained during several research expeditions and fisheries surveys conducted between 2006 and 2017 in the North East Pacific and Central and Eastern Atlantic. The North Pacific samples were collected by the Monterey Bay Aquarium Research Institute (MBARI), Moss Landing, CA-USA and the Northwest Fisheries Science Center (NWFSC), Newport, OR-USA. MBARI samples were collected by the remotely operated vehicles *Doc Ricketts* and *Ventana* using various collection

methods (respirometers, detritus and suction samplers). Sampling took place during the Midwater Ecology expeditions in the Monterey Bay Submarine Canyon from 2006 to 2016 and in the Gulf of California during 2012 and 2015 (Fig 2) (https://www.mbari.org/at-sea/expeditions/).

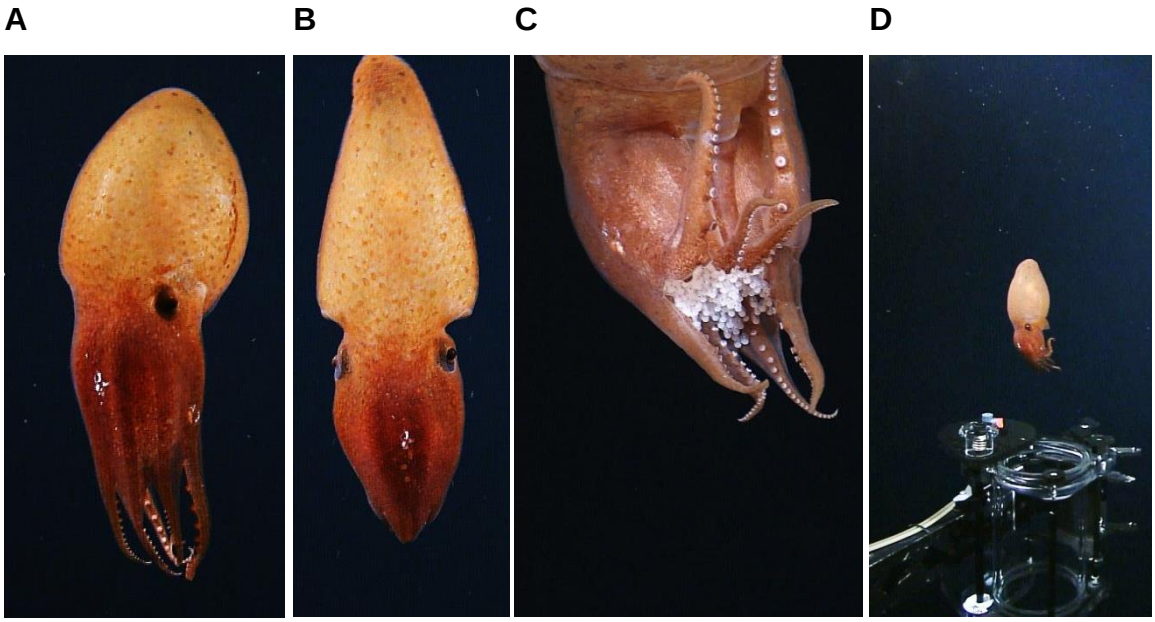

Fig 2. A sequence showing the capture of a female *Japetella diaphana* (ML 65 mm) brooding eggs at 945 m depth, during an expedition to the Davidson Seamount in January of 2006. **A-B**, mantle movement performed during swimming; **C**, a close on the egg clutch with developing embryos (~2-3 mm) inside the arm crown; **D**, the detritus sampler of the ROV *Ventana* is used to capture the specimen. Image credits: © 2006 MBARI.

Animals obtained by the NWFSC were captured as by-catch during the annual West Coast Groundfish Bottom Trawl Surveys (WCGBT) in 2013 and 2017 (Keller *et al.* 2017, NWFSC Groundfish Slope and Shelf Combination Survey 2018). Trawled specimens were captured along the west coast of the USA between 319 to 1206 m, and were frozen on board the vessels F/V *Excalibur*, F/V *Last Straw*, F/V *MS Julie* and F/V *Noah's Ark* (Fig 3, Table 1). Samples from the central Atlantic Ocean were collected during two German research expeditions in 2015. During March-April of 2015, the R/V *Walther Herwig III* cruise 383 (WH 383) to the eastern Atlantic captured animals by using a midwater trawl (‘Aalnetz’ or eel net) between 270 and 650 m (Table 1). The R/V *Maria S Merian* cruise 49 (MSM 49) collected samples in the vicinity of the Cape Verde archipelago during December 2015 (Christiansen *et al.* 2016). The collections were done using Multiple Opening Closing Net and

Environmental Sensing Systems (MOCNESS) (mouth openings of 1m^2 and 10m^2) between 400-1000 m.

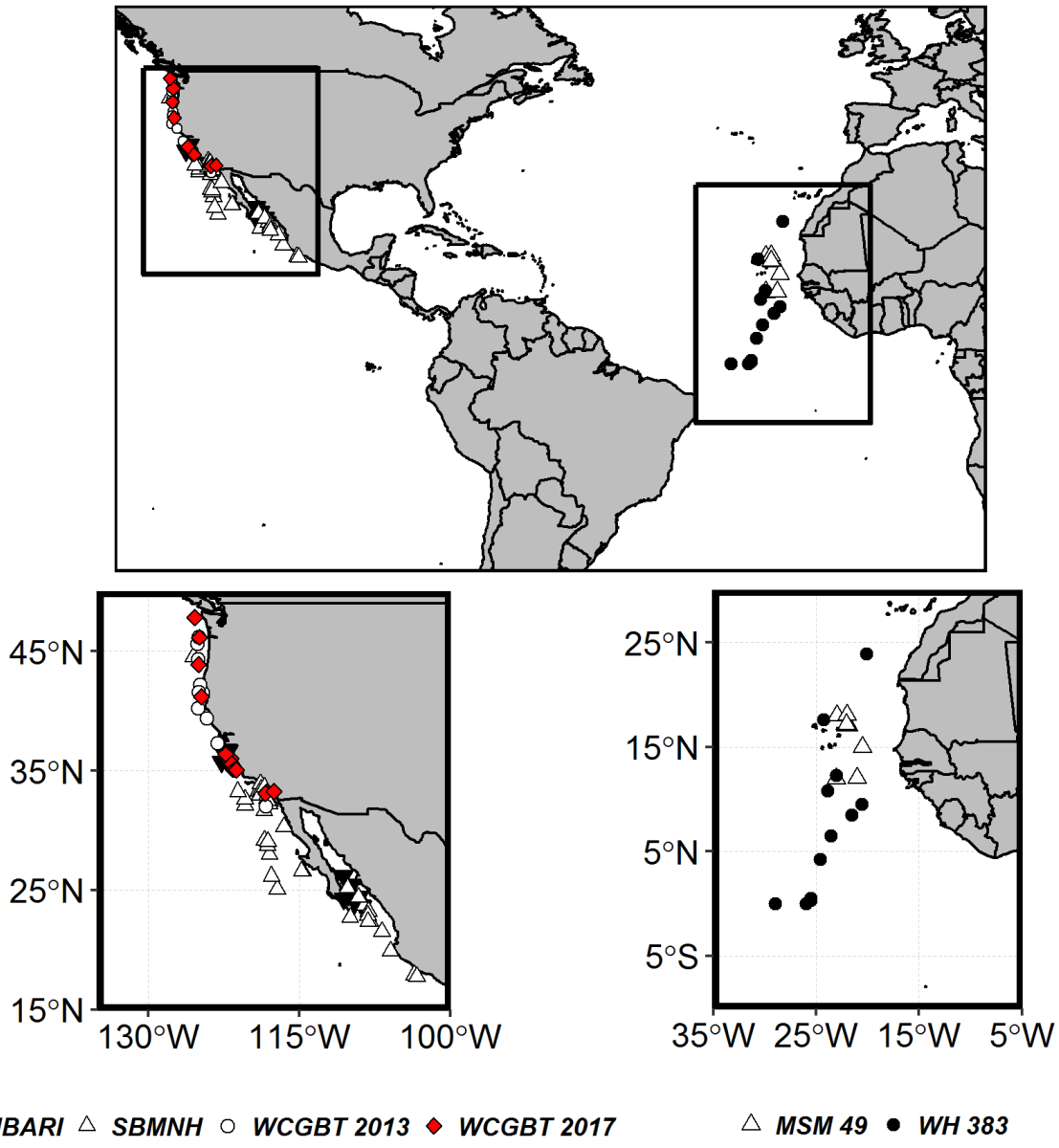

Fig 3. Capture locations of *Japetella diaphana* and *Vampyroteuthis infernalis*. Legend abbreviations: *MBARI* – Samples captured by the Monterey Bay Research Institute between 2006 and 2015; *SBMNH* – Santa Barbara Museum of Natural Sciences' samples captured between 1963-1995; *WCGBT* – samples captured during the West Coast Groundfish Bottom Trawl Surveys in 2013 and 2017; *MSM 49* and *WH 383*– Samples captured during the *Maria S Merian* 49 and *Walter Herwig III* 383 expeditions in 2015.

In order to assess the size range and maturity stages of *J. diaphana* and *V. infernalis*, we examined specimens from the collections of the Santa Barbara Museum of Natural History, Santa Barbara, CA-USA (SBMNH). The examined SBMNH specimens were collected between 1963 and 1995 off the California coast. The majority of specimens were collected during expeditions of the RV *Velero IV*, using Isaacs-Kidd Mid Water Trawl nets (IKMWT) at depths ranging from 457-3840 m.

Table 1. Summary of the examined specimens of *Japetella diaphana* and *Vampyroteuthis infernalis*. Collection abbreviations are explained in the methods; Sex: ♂ - male, ♀ - female, und – sex undetermined; Mantle Length (ML mm); Body Mass (BM g); Depth in meters; T - Temperature in °C; N - number of specimens; Beaks - beaks collected and examined.

Species	Collection	Sex	ML $_{mm}$ min-max(avr)	BM $_{g}$ min-max(avr)	Depth $_{m}$ min-max(avr)	T °C min-max(avr)	N	Beaks
J. diaphana	WH 383	und	9.8-34.3 (19.4)	0.1-3.2 (1)	270-650 (509)	5-12 (8)	13	13
		♂	20.9-29.2 (23.8)	0.7-4.6 (2)	412	7	3	3
	MSM 49	und	3.4-14 (8.8)	0.0-0.4 (0.1)	400-1000 (620)	5-10 (7.5)	10	6
		♂	18.6	0.8	800	6	1	1
		♀	13.4	0.2	800	6	1	1
	MBARI	und	25-72 (45.5)	2.5-39 (14)	783-1175 (895)	4-6 (5)	17	16
		♂	41-90 (69.8)	3.5-69.5 (38)	662-860 (795)	5	5	5
		♀	59-144 (92.5)	17.5-235 (93)	802-1402 (1015)	3-5 (4)	11	9
	WCGBT 2017	und	32.2-45 (39.3)	4.1-16.5 (11)	612-967 (805)	4-5 (4.5)	3	2
		♂	49	23.4	993	4.4	1	1
	SBMNH	und	7-73 (20)	0.1-38.4 (2)	1000-2926 (2493)	-	53	0
		♂	24-72 (47)	2.2-48.8 (15)	1000-2926 (1875)	-	20	0
		♀	19-93 (56)	0.8-101 (27)	457-3840 (2276)	-	47	0
V. infernalis	WH 383	und	28-63 (43)	4.6-34.2 (13)	415-580 (523)	7-10 (8.5)	6	4
	MSM 49	und	12-65 (27)	0.1-19 (4)	800-1000 (885)	5-7 (6)	7	7
		♂	20-44 (32)	1.1-18.4 (10)	900-1000 (950)	6	2	1
		♀	20-75 (49)	2-138 (58)	800-1000 (933)	6	3	3
	MBARI	und	46-136 (88)	2.2-285.7 (134)	653-1595 (1144)	3-5 (4)	5	5
		♂	89-120 (107)	142-207 (167)	581-1582 (1082)	3-5 (4)	3	3
		♀	95-115 (105)	70-259 (164)	767-809 (788)	5	2	2
	WCGBT 2013	und	57-103 (77)	23.2-107 (61)	909-1184 (1074)	3-4 (4)	6	6
		♂	64-114 (85)	24.2-125 (70)	874-1152 (1012)	3-4 (4)	10	9
	WCGBT 2017	und**	-	-	907	4	1	1
		♂	93-122 (106)	53-277 (121)	319-1206 (976)	3-7 (4)	10	9
		♀	65-102 (79)	37-70 (55)	498-1130 (895)	4-7 (5)	3	3
	SBMNH	und	26-38 (31)	4.3-8.3 (5.7)	1280-1829 (1500)	-	5	0
		♂	27-94 (62)	6.3-111 (45)	1143-3200 (2240)	-	17	0
		♀	31-89 (53)	10-149 (37)	1143-1829 (1533)	-	13	0
Total							**278**	**110**

***Animal was severely damaged but the beaks were intact.*

3.3.2 Biological measurements

A total 278 specimens were examined (185 *J. diaphana*; 93 *V*. *infernalis*). Most of the specimens belonged to the SBMNH collection (total = 155; 120 *J. diaphana*; 35 *V*. *infernalis*). Frozen specimens collected during research expeditions and fisheries surveys (N=123; 65 *J*. *diaphana*; 58 *V*. *infernalis*) were thawed in the laboratory and mantle length (ML), body mass (BM), sex and maturity stages were recorded. In severely damaged net-captured specimens, sex or maturity stages could not be determined and these specimens were treated as undetermined (Table 1). For twenty-four *J*. *diaphana* (ML 25-144 mm) and one *V*. *infernalis* (ML 124 mm) the beaks were collected but only the ML was recorded, no body mass. For these animals, BM was estimated using the relationship between ML and BM of frozen specimens:

J. *diaphana* - BM = $9.97e^{-05*}ML^{2.98}$; $p < 2\ e^{-16}$; $R^2 = 0.87$; N = 37 (eq. 1)

V. *infernalis* - BM = $1.19e^{-04*}ML^{2.99}$; $p < 1.3\ e^{-09}$; $R^2 = 0.71$; N = 50 (eq. 2)

The estimated parameters, standard errors, scatter plots, fitted values and residuals are provided in the appendix supplement S3 (Table S3; Fig S3).

3.3.3 Reproduction

Maturity stages were assigned based on the presence or absence of spermatophores in males, and macroscopic inspection of oocyte development in females. When sex could not be determined the specimens were assigned as juveniles (ML ≤ 25 mm). Males with a barely visible spermatophoric complex were considered immature. In maturing males spermatophores were absent or developing in the clearly visible spermatophoric complex. Males with enlarged suckers (*e.g.* ♂ *J*. *diaphana*) and fully developed spermatophores inside the Needham's sac or penis were considered mature.

The maturity stages of females were defined as: (a) immature, if reproductive organs were developing and ovaries had small and translucent oocytes (total length ≤ 2 mm); (b) maturing, if oocytes had a dark orange coloration and visible follicular folds; (c) mature, when females had a clearly visible circumoral photophore (Robison & Young 1981, Herring *et al.* 1987), and ripe oocytes that were dark orange/red in color (Fig 4). The gonadosomatic index was calculated as: GSI% = (GW/BM)*100, where GW is the total reproductive apparatus weight and BM the animal's total

weight in grams. Twelve females of *J. diaphana* (10 SBMHN, 2 MBARI) had the whole reproductive system dissected out and weighed. Their ovaries were opened, all oocytes were counted and a random subsample was measured using stereomicroscopy (magnification 6.3 to 25x). One brooding female collected by the MBARI in the Gulf of Mexico in 2015 had its ovaries and the developing clutch of eggs examined. Vampire squids' reproduction was investigated in previous studies and was not examined here (Pickford 1949, Hoving *et al.* 2015a).

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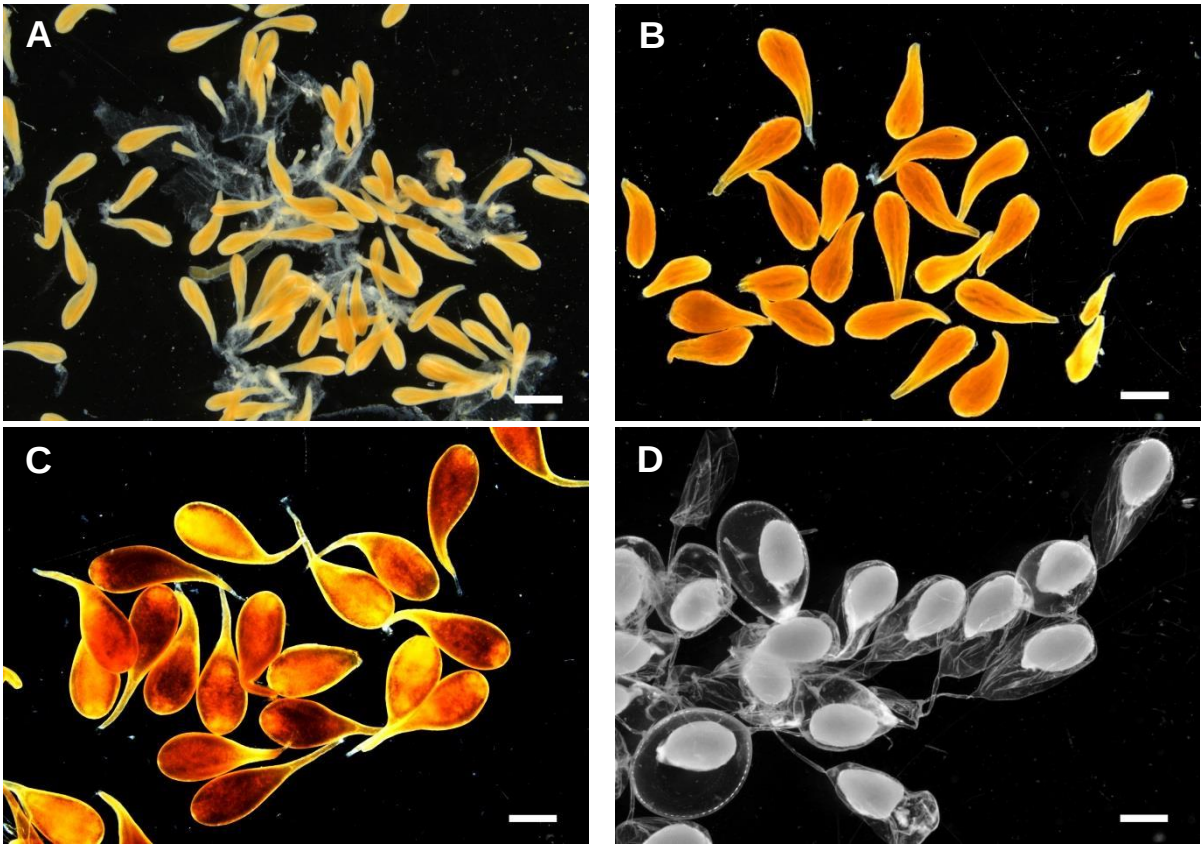

Fig 4. Different stages of oocyte development in females of *Japetella diaphana*. A - Immature developing oocytes; B - maturing striated oocytes; C - ripe oocytes; D - developing embryonic eggs from a brooding female. Scale bars = 1 mm.

3.3.4 Beaks sampling and preparation

The beaks were extracted from the buccal mass using scissors. Tissue remains were removed using clean wipes or by rubbing the beak with fingertips under running water. Clean beaks were placed in vials containing Milli-Q® water and stored in a refrigerator (~4 °C). Beaks from formalin preserved animals could not be removed without damaging the structure and could therefore not be used for growth-increment observation. The beak dimensions (beak length *BL* and hood length *HL*)

were measured following Clarke (1986). Beaks were prepared for growth-increment visualization using the methods described by Hernández-López *et al*. (2001) and Perales-Raya *et al.* (2010). The upper beaks were longitudinally sectioned in the middle, and growth-increments were quantified in the inner part of the beak lateral wall surfaces (LWS) following Perales-Raya et al. (2010).

The beaks of *J*. *diaphana* are lightly pigmented, with a light-brown coloration from the rostral tip to the middle of the crest. In these small and fragile semi-transparent beaks, growth-increments were best observed using transmitted light and by keeping the beak submerged in distilled water (Fig 5 A-B). Beaks of vampire squids are darkly pigmented and growth increments were best observed using a combination of transmitted and reflected light (Fig 5 C). The beak LWS were photographed using a stereo microscope camera (Leica MZ 9.5). The photographs were interlinked and the number of increments was quantified using Image-Pro Insight software. The total number of increments was counted twice for each specimen, and the agreement percentage between counts was assessed using the Coefficient of Variation and the Average Percent Error (CV and APE; equations described in chapter II) (Campana 2001, Ogle 2016).

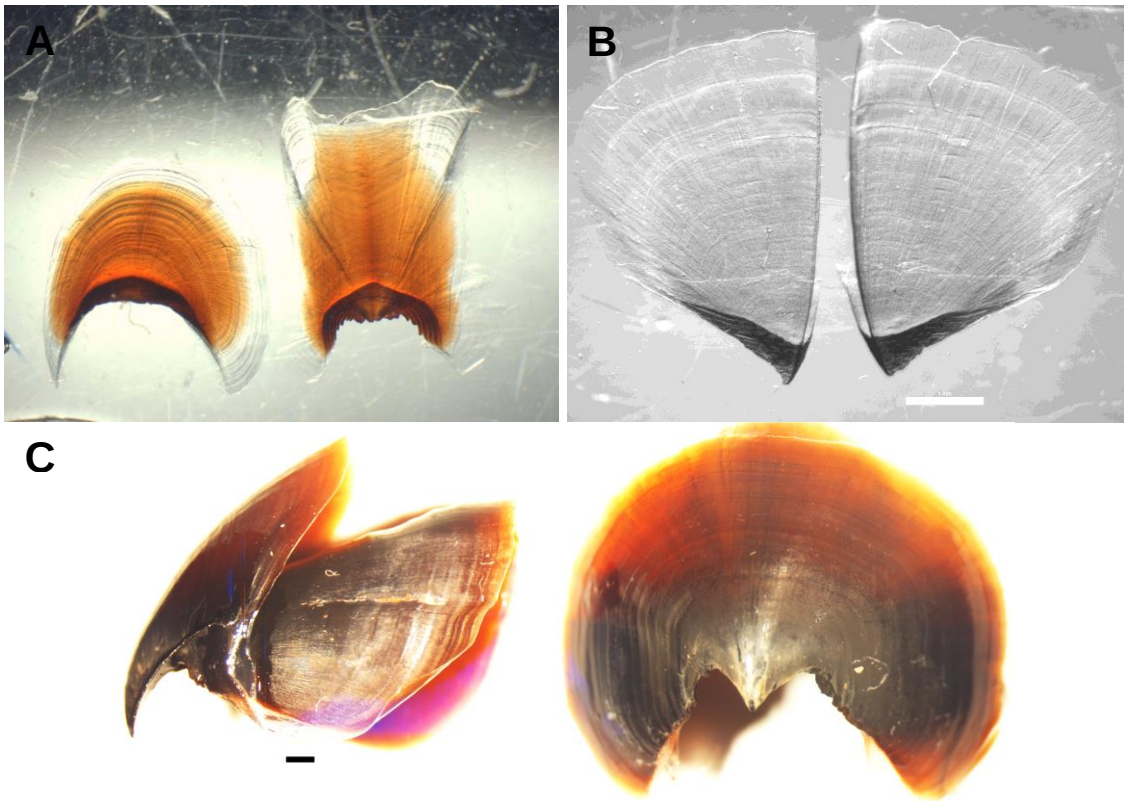

Fig 5. The beaks of *Japetella diaphana* and *Vampyroteuthis infernalis*. **A** - Upper (*left*) and lower (*right*) beak of *J*. *diaphana*; **B** - Sectioned upper beak of *J*. *diaphana* observed underwater using transmitted light; **C** – Upper (*left*) and lower (*right*) beak of *V*. *infernalis*. Scale bars represent 1 mm**.**

The mean value of the two increment counts was used to correlate the upper beak dimensions (BL and HL), body mass (BM) and number of increments. Both *J*. *diaphana* and *V*. *infernalis* are cephalopod species with a broad geographic distribution but difficult to obtain. Therefore, all analyses were made by grouping specimens captured during different years and locations. All statistical analysis were performed using R version 3.5 and RStudio (Rstudio Team 2015).

3.4 Results

3.4.1 Size and maturity

A wide range of sizes was examined in both species, with ML varying from 3.4 to 144 mm in *Japetella diaphana* (0.006-235 g BM) and 12 to 136 mm in *Vampyroteuthis infernalis* (0.08-286 g BM) (Fig 6). Most specimens captured during the expeditions to the Central and Eastern Atlantic were juveniles with ML less than 34 mm in *J*. *diaphana* and ML < 50 mm in *V*. *infernalis*.

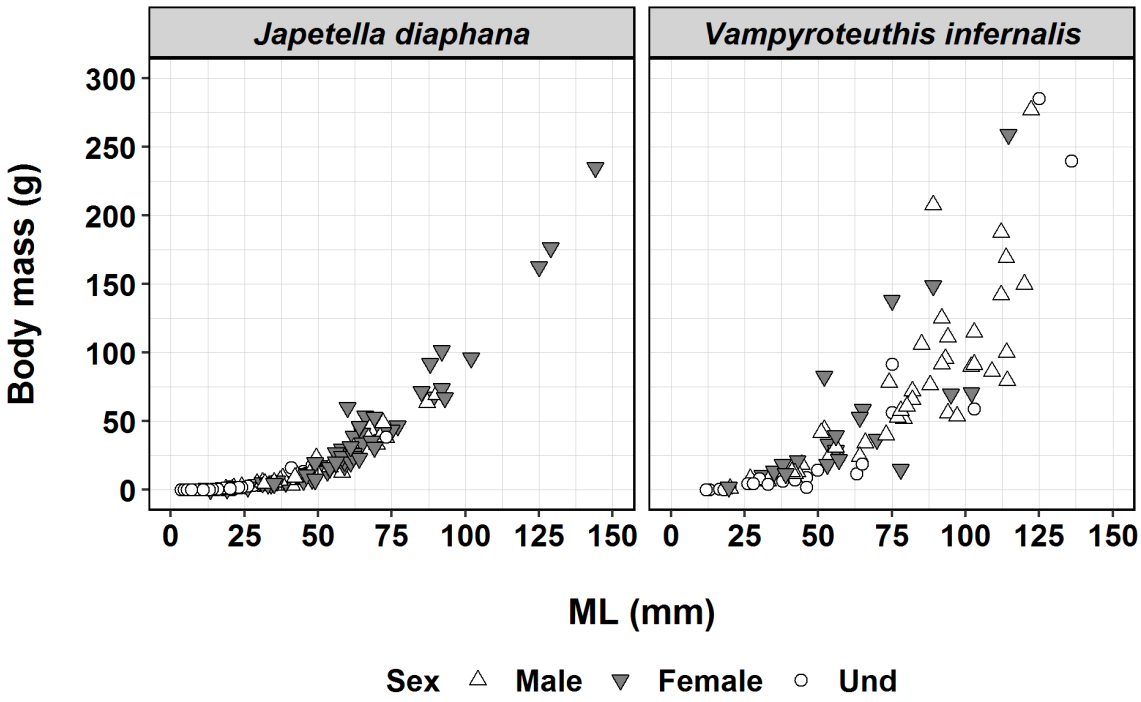

Fig 6. Relationship between mantle length and body weight of *Japetella diaphana* (N=184) and *Vampyroteuthis infernalis* (N = 87). *Und* - sex undetermined.

Size range within each maturity stage was also highly variable, especially among mature females of *J. diaphana* from North Pacific and Gulf of California (ML 53-144 mm, $\bar{x}$ = 89 mm; BM 18-235 g, $\bar{x}$ = 105 g) (Fig 7). In both species, sexual maturity occurs after animals reach mantle lengths between 50 and 70 mm. Only one male of *J. diaphana* (ML 73 mm, BM 38 g) was fully mature, with enlarged suckers on the third right arm and developed spermatophores aligned inside the spermatophoric complex. Two female *J. diaphana* had ovaries that contained only a few ripe oocytes and empty follicular folds and hence these females were considered spent (ML 54 and 56 mm; BM 16 and 20 g). The majority of the *V. infernalis* examined were juvenile or maturing specimens (N =53, ML 12-102 mm, BM 0.08-115 g), followed by mature males (N=24, ML 66-122 mm, BM 34-276 g), and only two mature females were examined (ML 75-89 mm; BM 138-149 g).

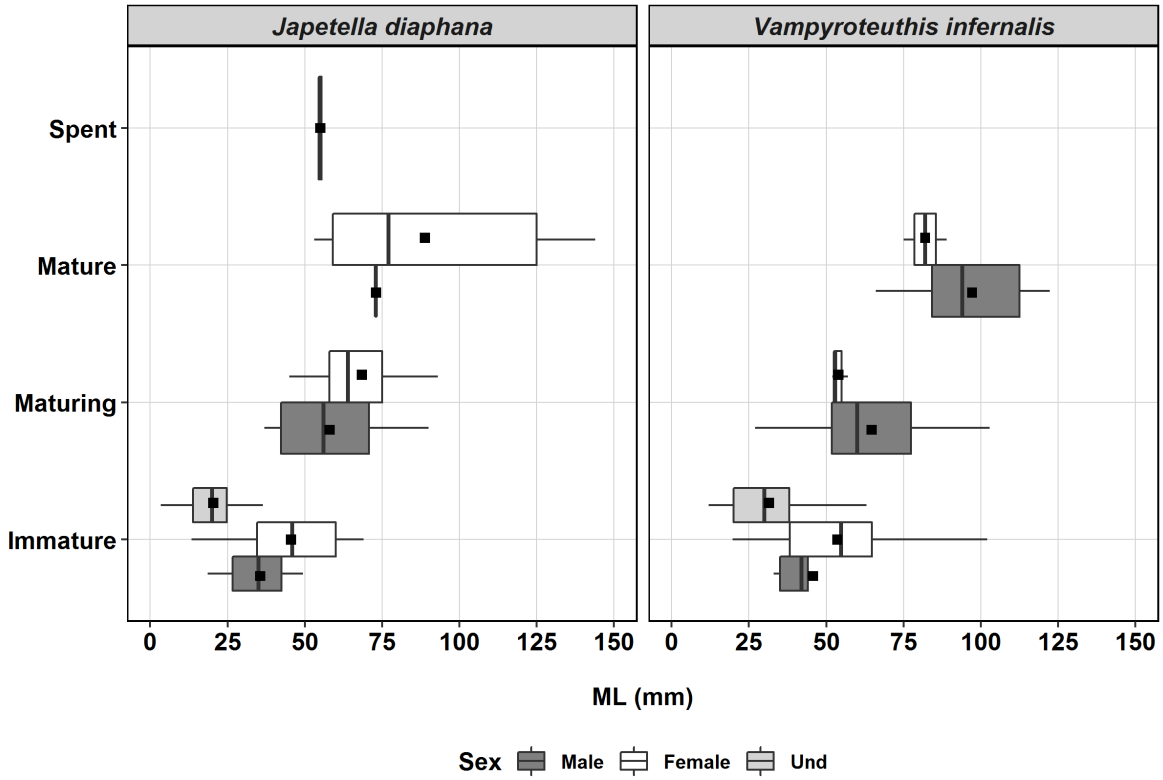

Fig 7. Size distribution at different maturity stages of *Japetella diaphana* and *Vampyroteuthis infernalis*. Vertical bars represent size range less than 5 mm; Black squares are mean values. Lines inside boxes represent the median, box size represents 1st to 3rd quartiles, and whiskers extend to 1.5x IQR.

The size distribution observed in the museum specimens suggests that the population structure is complex and animals of all sizes and maturity stages can be captured throughout the year. For example, in January of 1970 the R/V *Velero IV* captured at 2706 m 10 specimens of *J. diaphana* with ML varying from 16 to 48 mm (4 males, 2 females and 4 undetermined; Catalog SBMNH 471374). Their maturity stages ranged from juvenile to maturing, with the most mature male already exhibiting enlarged suckers (Fig 8).

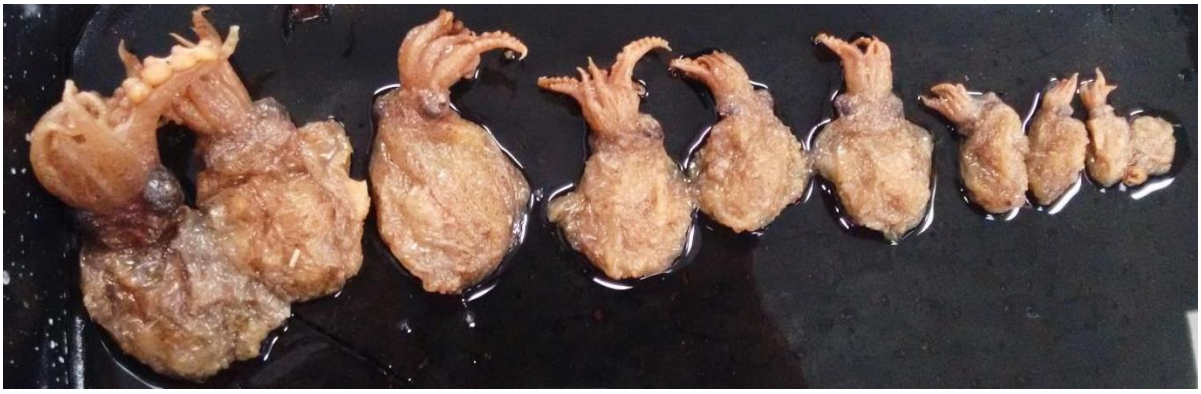

Fig 8. Specimens of *Japetella diaphana* examined at the Santa Barbara Museum of Natural History (Catalog number SBMNH 471374). The first animal on the left is a maturing male with enlarged suckers on the third right arm.

The twelve *J*. *diaphana* females for which ovaries were examined in detail represented all maturity stages. The initial stage of oogenesis when spherical oocytes start to be formed was not observed in any of the examined females. Ovary weights in relation to body mass (GSI *%*) ranged from less than 1% in immature females to as much as 10% of BM in mature specimens (Table 2).

Table 2. Oocytes total number and size frequency distribution in different maturity stages of twelve *Japetella diaphana*. N - specimens examined; ML - mantle length (mm); BM - body mass (g); GSI - gonadosomatic index; Oocyte $N.g^{-1}$ - number of oocytes per gram; Values given in ranges and mean inside parentheses.

Maturity stage	N	ML (mm)	BM (g)	GSI (%)	Number of Oocytes	Oocyte $N.g^{-1}$	Oocyte length (mm)	Oocytes measured
Immature	4	59-66 (63)	21-54 (37)	0,4-0,9(0,6)	659-4051 (2167)	32-75 (54)	0,3-1,8 (1)	742
Maturing	4	56-88 (67)	27-92 (49)	1,5-7 (4)	1186-2716 (1781)	26-68 (42)	0,4-3,9 (2,7)	798
Mature	3	53-89 (67)	18-68 (37)	4-10 (8)	1648-2208 (1933)	29-124 (71)	0,8-4,4 (2,6)	992
Brooding	1	59	17.5		1419*		2.1-2.9 (2.5)†	20

* Number of embryonic eggs that the female was brooding; † Length of the corion longest axis, without stalks.

The ovaries in immature specimens of *J. diaphana* had as much as 4051 semi-transparent elongated oocytes ≤ 1.8 mm, while oviducal glands were small and creamy white. Maturing ovaries had from 1186 to 2716 oocytes with visible longitudinal follicular folds (oocyte length 0.5-3.9 mm, $\bar{x}$ = 2.7 mm), and well-formed oviducal glands of beige color. Mature ovaries were swollen and had enlarged dark brown oviducal glands. Oocytes number in mature ovaries ranged from 1648 to 2208, with lengths varying from 0.8 to 4.4 mm. Maturing and mature females also had a variable number of atretic oocytes (N = 6 to 1176) measuring from 0.4 to 2.7 mm. Four females of *J.* diaphana in different stages of oogenesis were selected to illustrate the oocytes length distribution (Fig 9).

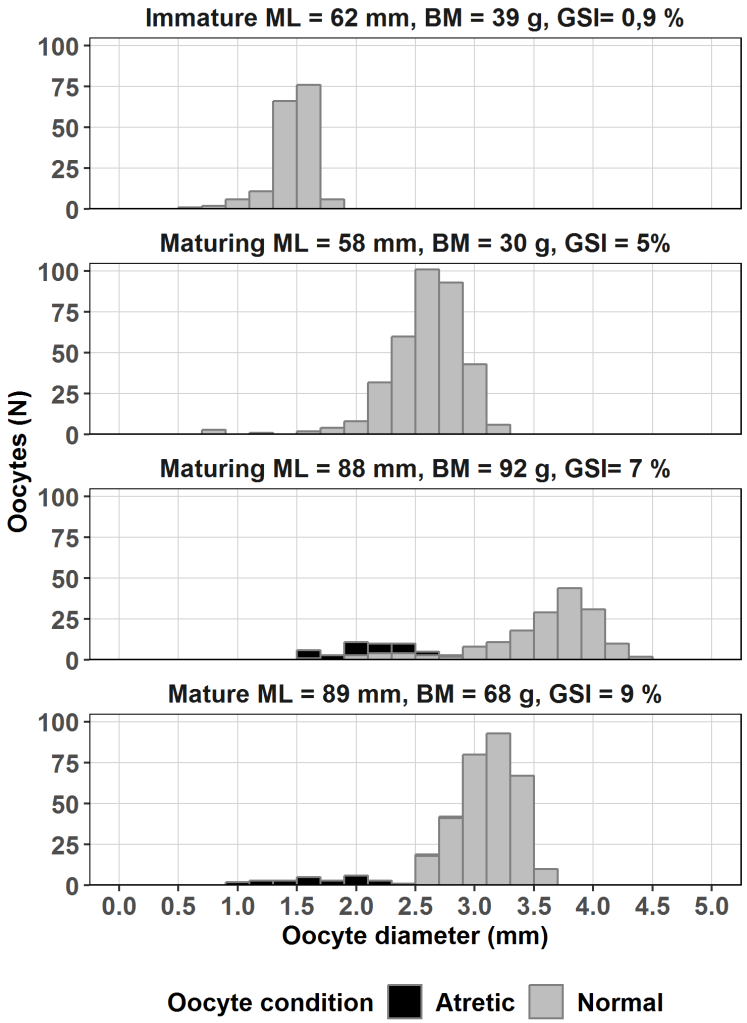

Fig 9. Oocyte length–frequency distribution at different maturity stages of four *Japetella diaphana* females.

The most advanced reproductive stage was observed in a brooding female captured at 1352 m during an MBARI's expedition to the Gulf of California in 2015. This specimen was significantly smaller than other mature females (ML 59 mm; BM 17.5 g). Inside the arm crown, she was holding a clutch with 1419 eggs in pre-organogenetic stage (corion $\bar{x}$ = 2.5 x 1.7 mm; embryos $\bar{x}$ = 1.5 x 0.9 mm). After preservation in formalin and storage in alcohol the eggs weighed in average 3.3 mg (Fig 4). Inside the ovary she had two ripe eggs which were 3.2 mm long plus 0.8 mm stalks.

3.4.2 Beak growth and number of increments

For 110 specimens the growth increments in upper beak LWS were quantified (57 *J. diaphana*, 53 *V. infernalis*). Overall, growth increments are regular and easy to

discern in both species. Increments in the translucent beak lateral walls of *J. diaphana* were best viewed using the stereomicroscope's transmitted-light, and by creating contrast by moving the mirror that is situated beneath a transparent stage (Fig 10). The same procedure was applied in beaks of juvenile specimens of *V. infernalis*. However, maturing animals have larger, darkly pigmented beaks and transmitted light does not pass through the structure easily. In these cases a combination of transmitted and reflected light was used. In vampire squid, the beak's most pigmented area is the anterior portion near to the rostrum. This dark and opaque region is where the first increments are deposited, and increments could be observed only using reflected light.

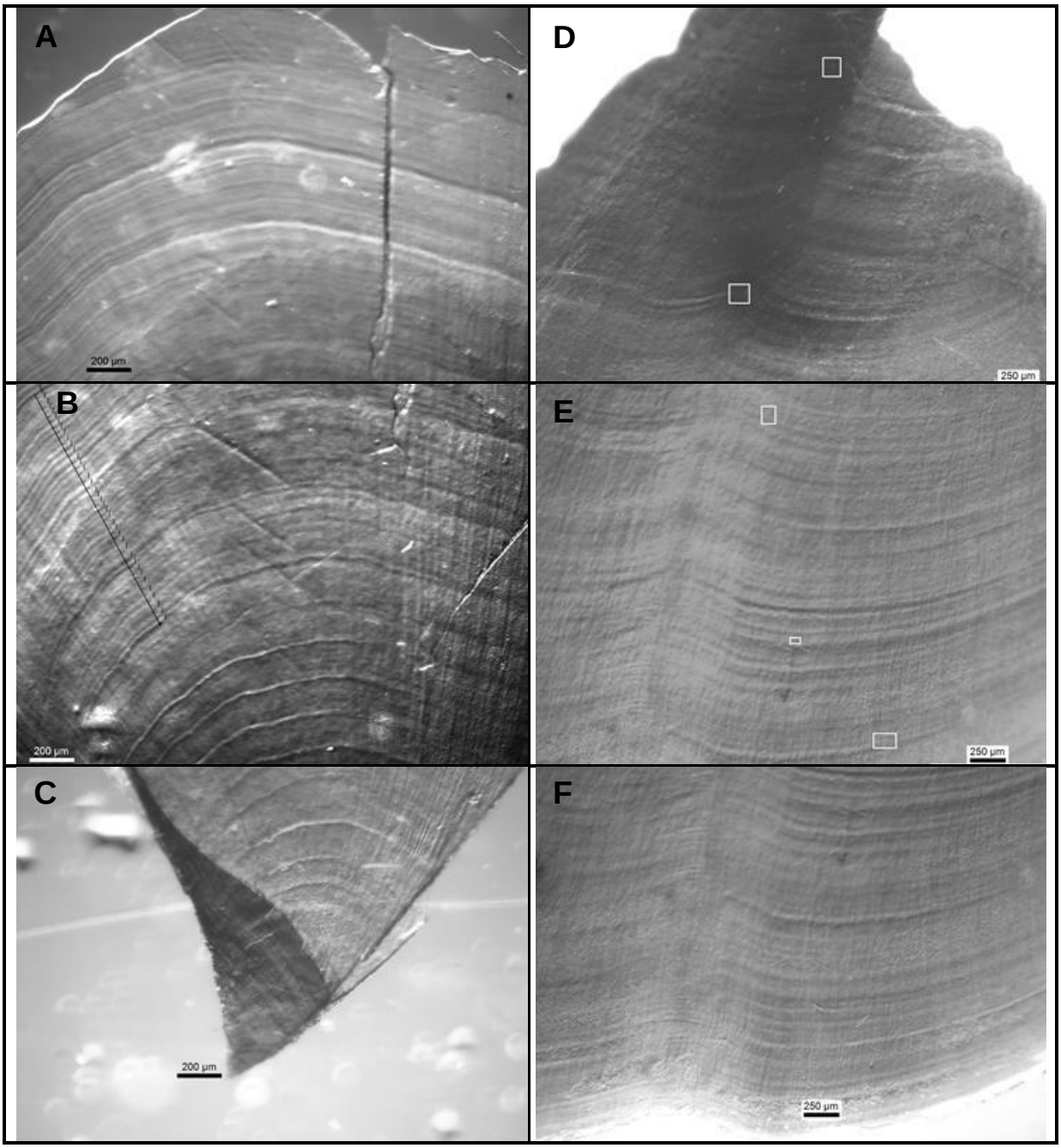

Fig 10. Growth increments in the inner surface of upper beaks lateral walls of *Japetella diaphana* (**A-C**) and *Vampyroteuthis infernalis* (**D-F**). **A** - posterior region of LWS were the last increments are deposited; **B** - medial region with a black line showing how first-order increments are enumerated and eight second-order growth bands; **C** - anterior region where the first increments are deposited; **D-F**, schematic view from the anterior to the posterior regions of an upper beak of *V. infernalis*. White squares are reference points for linking several photographs.

The precision between the two counts was high, with mean CV and APE values less than 3% (*J*. *diaphana* CV=2.2, APE=1.56, N=57; *V*. *infernalis*, CV=2.4, APE=1.68, N=53). The second counting usually resulted in a higher number of increments and it was used to calculate growth-increment widths (*Winc*). The *Winc* values were highly variable in beak LWS for both species, but mean widths were similar in all regions (Table 3). Mean increment width was slightly larger in *V*. *infernalis* (*Winc* 14-98; $\bar{x}$ = 42 µm) than in *J*. *diaphana* (*Winc* 10-91; $\bar{x}$ = 35 µm). Second-order growth bands (*i.e.* check marks) formed by a variable group of first-order increments were observed in both species. These check marks were more easily recognizable in *J. diaphana* (Fig 10 A-C), however their frequency and regularity was not assessed in further detail.

Table 3. Growth-increment widths (*Winc*) range in different regions of the beak lateral walls of *Japetella diaphana* and *Vampyroteuthis infernalis*; Standard deviation (*SD*), median and interquartile range (IQR) are given.

Species	Beak region	*Winc* (µm)	*SD*	median	IQR
J. diaphana	Anterior	10-91 (34)	13.42	31	18
	Medial	10-77 (36)	12.37	35	16
	Posterior	10-85 (35)	12.23	34	15
V. infernalis	Anterior	14-84 (41)	10.93	40	15
	Medial	14-90 (43)	9.96	42	13
	Posterior	16-98 (42)	12.63	40	15

3.4.2.1 *Japetella diaphana*

The mean number of growth increments (*Ginc*) after two counts in *J*. *diaphana* varied from 21 in the smallest specimen (ML 4.5 mm; BM 1.23 mg) to 207 in a mature female (ML 144, estimated BM ~235 g). Maturing specimens had between 119 and 173 growth increments, with a mean of 142 for males and 145 for females. For most specimens neither sex or the maturity stages could be determined and the number of increments was highly variable in animals with a mean BM less than 25 g (Table 4). The beaks of immature animals that weighed less than 18 g had as much as 157 growth increments. The brooding female captured by the MBARI in 2015 had a mean of 197 increments (192-202). The beaks of the brooding female captured in 2006 were not available for examination.

Table 4. Number of growth increments in different sexes, maturity stages and size range of *Japetella diaphana* and *Vampyroteuthis infernalis*. All values are given in ranges: maximum-minimum (mean).

Species	Sex	Stage	ML mm	BM g	Increments	N
Japetella diaphana	Male	Immature	19-49 (28)	0.7-23.4 (6.2)	73-146 (104)	5
		Maturing	58-90 (78)	12.9-69.5 (48.7)	128-156 (142)	3
		Mature	73	38.2	99	1
	Female	Immature	13.4	0.2	58	1
		Maturing	60-102 (82)	43.4-96.2 (68.3)	119-173 (145)	4
		Mature	59-144 (104)	17.5-235 (117.7)	120-207 (162)	6
	Und	Immature	4.5-48 (24)	0-18. 1 (3.4)	21-157 (75)	30
		Und	41-72 (55)	12.2-39 (22.3)	63-158 (115)	7
Vampyroteuthis infernalis	Male	Immature	44-93	18.4-95.8	235 - 262	2
		Maturing	64-103 (86)	24.2-115 (54.4)	238-334 (272)	5
		Mature	82-122 (102)	56-277 (128.4)	258-375 (305)	15
	Female	Immature	20-102 (62)	2-701 (40.2)	158-297 (250)	5
		Mature	114-75	259-138	326-375	2
		Und	95	70	331	1
	Und	Immature	12-63 (32)	0.1-12 (4.8)	89-308 (167)	12
		Und	57-136 (91)	18.8-285.7 (101)	238-373 (293)	11
					Total	**110**

The relationship between *Ginc* and BM was best described by a power curve ($BM = 3.19e^{-5} * Ginc^{2.87}$; R^2=0.55; $p < 0.001$; N=57) (Fig 11A). However, the data was scattered with many specimens of less than 25 g having as much as 125 increments, while a mature female (ML 129, BM 177 g) had only 120 increments. The relationships between BL, HL and BM were best described by power and linear models, respectively. The best model to predict *Ginc* from beak lengths (BL) was also allometric, and it provided the best fit to the data among all comparisons made ($Ginc = 46.02 * BL^{0.65}$; R^2=0.87; $p < 0.001$; N=54). All tested models and the associated estimated parameters are presented in Table 5.

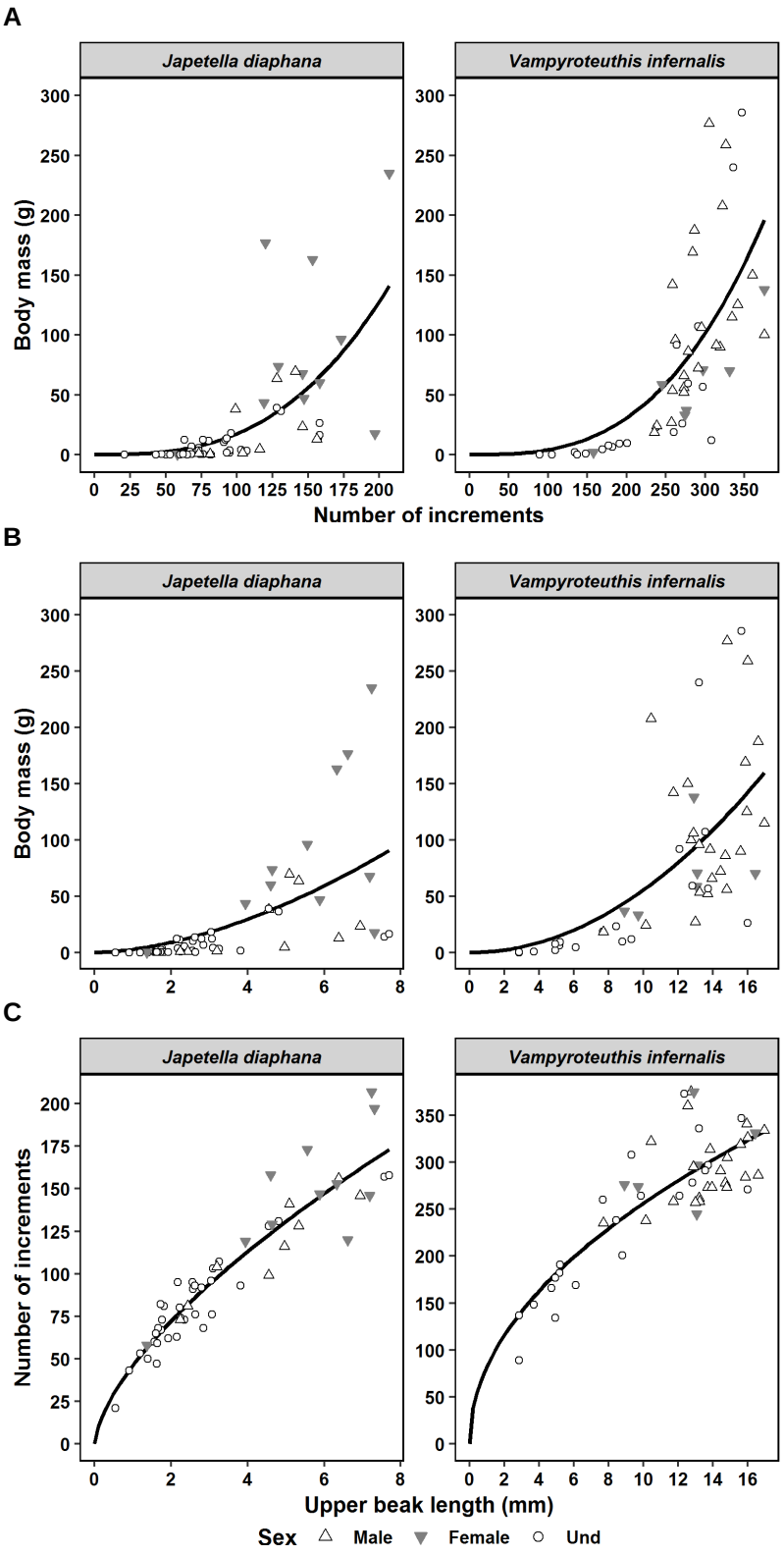

Fig 11. Scatter plots of different relationships between body mass (BM g), number of increments and beak length (BL mm) for *Japetella diaphana* and *Vampyroteuthis infernalis*. A - Number of increments in beak lateral walls and body mass (g); B – Relationship between upper beak length (BL mm) and body mass (g); C – Relationship between length (BL mm) and number of growth increments in the upper beaks. Solid lines in all plots represent the best fit using the power model $y \sim ax^b$. Parameter estimates in Table 5.

3.4.2.2 *Vampyroteuthis infernalis*

A higher number of growth increments was observed in the beaks of *V. infernalis* compared to those of *J. diaphana* (Table 4). The smallest juvenile was captured during the MSM 49 cruise at ~600 m, and it had a mean of 89 increments (ML 12 mm; BM 0.22 g). The number of increments was highly variable among immature specimens, ranging from 89 to 308 ($\bar{x}$ = 198). Maturing animals had between 238 and 334 increments ($\bar{x}$ = 272) and the most mature between 258 and 375 increments (Table 4). Two specimens had a *Ginc* of 375; one was a mature male (ML 114, BM 100 g) captured in the North Pacific at a bottom depth of 1019 m during a WCGBT cruise in 2013, and the other was a mature female captured at 800 m during the MSM 49 cruise in 2015 (ML 75, BM 138 g). This mature female had signs of a recent spawning event, since its ovary contained many developing oocytes but also four ripe eggs measuring 3.2 x 2.9 mm. The largest mature female examined (ML 124, BM 398 g) had 326 increments in the beak LWS.

Similar to what was observed in *J. diaphana*, the relationships between beak dimensions (BL and HL), *Ginc* and BM were best described by power curves (Table 5). The relationship between *Ginc* and BM was highly scattered, and although the correlation is significant and positive ($BM = 5.11e^{-6}*Ginc^{2.95}$; R^2=0.47; $p < 0.001$; N=50), the power curve overestimates the body weight for specimens with less than 200 increments and underrestimates the weight of specimens with more than 250 increments. The model that predicts number of *Ginc* from BL ($Ginc = 81.76*BL^{0.5}$; R^2=0.70; $p < 0.001$; N=51) was also the best described by a power curve, and it seems that the number of increments tend to estabilize towards the end with larger beaks depositing increments under a lower rate (Fig 11 C).

Table 5. Estimated parameters of different relationships between body mass (g), beak length (BL mm), hood length (HL mm) and number of growth increments (G*inc*) in upper beaks of the species. Relationships are presented as *y ~ x*. Akaike's information criterion (AIC) and R^2 values of the fitted models are given; Linear: *y ~ a + b*x*; exponential: *y ~ a*exp(b*x)*; power: $y \sim a*x^b$. Best fits are in italics.

Species	Relationship	Model	*a*	*b*	*AIC*	R^2	N
J. diaphana	G*inc* ~ BM	Linear	-44.86	0.702	575	0.38	57
		Exponential	2.35	$2.04e^{-2}$	572	0.43	57
		Power	*$3.19e^{-5}$*	*2.87*	*572*	*0.55*	*57*
	BL ~ BM	Linear	-24.58	14.43	549	0.38	52
		Exponential	6.74	$3.41e^{-1}$	553	0.31	52
		Power	*$9.57e^{-2}$*	*3.46*	*549*	*0.70*	*52*
	HL ~ BM	*Linear*	*-20.63*	*41.42*	*561*	*0.34*	*53*
		Exponential	9.93	0.80	569	0.24	53
		Power	22.08	1.42	563	0.32	53
	BL ~ G*inc*	Linear	33.88	18.86	453	0.86	54
		Power	*46.02*	*0.65*	*448*	*0.87*	*54*
V. infernalis	G*inc* ~ BM	Linear	-118.72	0.75	550	0.45	50
		Exponential	5.35	$9.68e^{-3}$	551	0.40	50
		Power	*$5.11e^{-6}$*	*2.95*	*548*	*0.47*	*50*
	BL ~ BM	Linear	-56.41	11.95	534	0.39	48
		Exponential	10.61	0.16	5535	0.37	48
		Power	*0.55*	*2. 00*	*533*	*0.50*	*48*
	HL ~ BM	Linear	-53.91	15.80	521	0.41	47
		Exponential	10.2	0.22	522	0.41	47
		Power	*$3.92e^{-2}$*	*3.33*	*520*	*0.50*	*47*
	BL ~ G*inc*	Linear	115.68	13.34	522	0.66	51
		Power	*81.76*	*0.50*	*516*	*0.70*	*51*

3.5 Discussion

The 278 specimens examined here provided a good overview on size and maturity of *J. diaphana* and *V. infernalis*. Both species were captured within the known depth and temperature ranges previously reported by other authors (Pickford 1946, Thore 1949, Roper & Young 1975, Young 1978). The juveniles (ML ≤ 35 mm) that were captured during the MSM49 and WH383 expeditions in the Eastern and Central Atlantic were encountered in relatively shallower and warmer waters (400-1000 m, 5-12°C), compared to the specimens from the North Pacific (600-2900 m, 3-7°C). Interestingly, most of the hatchlings and early juveniles examined by Thore (1949) were captured in water depths less than 300 m and temperature regimes above 12°C, which corresponds with the upper range of our samples. However, since our animals were captured with different collection methodologies during different seasons, years and in different oceanic regions, compared to Thore (1949), a detailed objective comparison of distribution patterns is not possible.

The range of sizes and maturity stages of the examined specimens suggest that reproduction and hatching in both species are non-seasonal, and individuals may originate from several different cohorts. Thore (1949, p.10) states in his monograph that "*When many specimens are taken in one haul, all are of about the same size*… *that makes it probable that (Japetella) diaphana most often lives in shoals, in all probability corresponding to hatches of young from the spawning of one or a few females*". The lots examined at the SBMNH often had specimens of different size classes and maturity stages, from early juveniles to mature, which were collected in the same trawl haul. This wide size range is in line with Thore his findings. However, it is unlikely that *Japetella* occurs in shoals. The examined *Japetella* that were observed and captured by submersibles in the Gulf of California and Monterey Bay always occurred solitary and never in groups as suggested by Thore (1949).

Mature specimens of *J. diaphana* had mantle lengths that exceeded 50 mm, corresponding to observations reported by Young (1978) and Thore (1949). Females of *J. diaphana* exhibited synchronous ovulation and the potential fecundity of mature specimens was estimated to be ~2000 eggs. The size-frequency distribution of the oocytes is unimodal and the potential fecundity in immature and maturing females (~4000 oocytes) is higher than the number of eggs found in in mature specimens. This indicates that many of the initial oocyte stock ceases to develop at oocyte length of ~1.5-3 mm and then degenerate or resorb, a process observed in other cold water octopods (Laptikhovsky 2001, 2013). Resorption of oocytes can happen if there is competition for space within the ovary or could be a strategy to mobilize energy when food consumption cannot support the development of the full oocyte stock (Mangold 1987, Boyle & Chevis 1992). The potential fecundity of *J. diaphana* is much lower than the fecundity observed in members of epipelagic Argonautoid octopod families (e.g., Argonautidae, Ocythoidae and Tremoctopodidae) which have potential fecundity between 85,000 and 300,000 eggs (Hochberg *et al.* 1992, Laptikhovsky & Salman 2003, Jereb *et al.* 2016).

Observations on the eggs, hatchlings and brooding behaviour in bolitaenids have been previously reported (Thore 1949, Young 1972, 1978), but the potential fecundity and egg batch size are unknown. The brooding female examined here was captured in the Gulf of California at 1352 m (water temperature 3.4°C). The female was carrying a clutch of 1419 eggs which were in the pre-organogenetic stage and

carried inside the arm crown. The eggs were held in front of the mouth and likely prevented feeding. This specimen (ML 59 mm, BM 17.5 g) was considerably smaller than the average body size of other mature females (ML 89 mm, BM 105 g) examined here, which may suggest that the body tissue is broken down and used as an energy source during the extended brooding times. Similar tissue breakdown has been observed for brooding gonatid squids, and mature/spent onychoteuthid squids (Jackson & Mladenov 1994, Seibel *et al.* 2000, 2005). Females of benthic octopods can lose between 25% to 70% of the body weight while brooding eggs (Anderson *et al.* 2002). The energetic costs associated with such strategy and the size of the egg clutch observed, suggest that all eggs are released as a single batch, there is a single spawning event which is associated with brooding period and after brooding the female dies. The females of pelagic Argonautoid octopods also provide parental care to their young (Finn 2016). In the ovoviviparous species *Ocythoe tuberculata* the eggs develop in the female oviducts until hatching, while in *Argonauta* spp. the eggs are carried inside the brood shell and *Tremoctopus violaceus* broods its eggs attached to a pair of calcified 'egg bases' on the dorsal arms (Laptikhovsky & Salman 2003, Finn 2016). Synchronous spawning with brooding is also known for gonatid and bathyteuthid squids which brood their eggs in the bathypelagic zone (Seibel *et al.* 2000, 2005, Bush *et al.* 2012).

Since *J. diaphana* embryos measured ~ 1.5 mm and hatchling size is about ML 2.5 mm (Hochberg *et al.* 1992), it is likely that embryos would require a considerable time to fully develop while the female cares for the eggs. Seibel *et al*. (2000) estimated that the eggs of the squid *Gonatus onyx*, with egg sizes similar to those of *J. diaphana*, require nine months to develop while the female cares for them in the bathypelagic zone (1.7−3.0°C). However, the authors estimated this embryonic development time based on a limited dataset of seven species of squids and octopods. Robison *et al*. (2014) published a mathematic model that predicts embryonic development based on the environmental temperature associated with the life cycle of 22 octopod species. These data included direct *in situ* observations of brooding deep-sea octopus *Graneledone boreopacifica* which lasted for 53 months (Robison *et al.* 2014). The model estimates that *J. diaphana* eggs may require 731 days to complete embryogenesis at average environmental temperatures of 4.5 °C (Schwarz *et al.* 2018). Such long embryonic periods are not uncommon for marine mollusks (e.g. bivalves and gastropods) living in polar temperatures < 5 °C, and the

embryos of some of these species require as much as 25 months to hatch (Hain & Arnaud 1992, Pörtner *et al.* 2007, Peck 2016).

Vampire squids become sexually mature at sizes similar to those of *J. diaphana*, and females can undergo more than one reproductive cycle (Hoving *et al.* 2015a). The sizes at maturity we found for males and female vampire squid are similar to those reported by Pickford (1949). Vampire squid males become sexually mature after attaining a ML ~60 mm and the few individuals we examined suggest that females mature when their ML exceeds 75 mm. Hoving *et al*. (2015a) examined adult females with ML ranging from 89 to 132 mm, indicating that the animals can continue to grow between reproductive cycles. It is worth noting that some of the large males we examined at the SBMNH had no visibly developed spermatophores. It is possible that like in females, males were in a reproductive resting phase, and in between reproductive events. This observation was also made by Young (1964, p.157) who stated: "*Within the male there is a cyclic development of the gonads and accessory structures, a situation that, to my knowledge, is unique among cephalopods.*" The author noted that a large male (ML 102 mm) had a completely developed prostate gland and an undeveloped penis, while in smaller specimens the reproductive system was fully developed. These observations deserve further investigation.

The number of growth increments (*Ginc*) in the beak LWS varied from 21 to 207 in *J. diaphana* and from 89 to 375 in *V. infernalis*. Assuming daily increment deposition, this would indicate a lifespan that does not exceed 1-2 years. If beak increments were deposited daily, the size extremes for the specimens of *J. diaphana* examined here (BM 235 g, *Ginc* 207; BM 0.0123 mg, *Ginc* 21), would result in a *G* value of 5.3% BM day^{-1} (for *G* equation see chapters I and II). This *G* value is similar to those of the fastest growing neritic benthic octopods living at 18-25°C (Schwarz *et al.* 2018), and hence seems to be unrealistic. The spawning female vampire squid that we examined (ML 75, BM 138 g) had 375 increments. If we assume daily deposition of growth increments, this specimen would be about one year old. However, the ovary of this specimen was full of developing oocytes and she would potentially continue to grow and spawn. Adult vampire squid females can undergo several spawning events after reaching maturity, and some specimens previously examined at the SBMNH weighed as much as 500 g after preservation (Hoving *et al.* 2015b). Vampire squid can reach BM of 1050 g (Seibel *et al.* 1997), indicating that

adults might need a considerable time to grow beyond the sizes we examined here (< 300 g).

Increment deposition in beaks has been validated to be daily in tropical and temperate octopod species (Rodríguez-domínguez *et al.* 2013, Bárcenas *et al.* 2014, Perales-Raya *et al.* 2014). However, the rate of growth-increment deposition may be positively correlated to environmental temperature, and animals exposed to lower temperature regimes might need more time to deposit growth increments (Perales-Raya *et al.* 2018). There is evidence that physiological processes may happen at a reduced pace in deep-sea and cold water invertebrate species (Hain et al. 1992, Wells & Clarke 1996, Young 2003, Peck et al. 2006). The time required to ingest and digest food, excrete and growth in marine invertebrates living in water temperatures below 5°C can take 2-5 times longer than in those living at 10-15°C (Peck 1998, 2016). Hence the deposition of new chitin layers in cephalopod beaks, which result from the metabolic and secretory activity of cells distributed in the buccal mass (Dilly & Nixon 1976), may be slower in cold-water animals with low metabolic rates.

What both *J. diaphana* and *V. infernalis* have in common is the extremely low mass specific metabolic rate which is comparable to jellyfish (Thuesen & Childress 1994, Seibel *et al.* 1997). When metabolic rates derived from mass-specific oxygen consumption (M_{O2} as µmol O2 g^{-1} h^{-1}) are compared between adults of *J. diaphana* (BM 153 g, M_{O2} = 0.06), *V. infernalis* (BM 30-225 g, M_{O2} 0.075-0.035), and benthic neritic octopods, the differences indicate that their pace of life should be slower than that of temperate and tropical neritic octopods (Seibel 2007, Table S2). The metabolic rates of *J. diaphana* and *V. infernalis* are six to tenfold lower than that of adults of the Antarctic octopods *Pareledone charcoti* (BM 136 g, M_{O2} = 0.45) and as much as 32 fold lower compared to a juvenile of the tropical species *Octopus maya* (BM = 45 g, M_{O2} = 2.45). Growth increments in beaks of *O. maya* were validated to be deposited on a daily basis for juveniles with BM 4-100 g and 63-122 days old (Bárcenas *et al.* 2014), and there are strong indications that increments need more than one day to be deposited in *P. charcoti* (see chapter 2, Schwarz *et al*. 2019). The number of growth increments in the beaks' rostral sections of ommastrephid squids correlate well with the number of increments found in statoliths, and increments in both structures are assumed to be deposited daily (Liu *et al.* 2015). However, metabolic rates of oceanic ommastrephid squids can be as much as 140 times higher than those of *V*. *infernalis* (Baldwin 1982, Seibel *et al.* 1998). Moreover, the metabolic

rates of *Nautilus pompilius*, a cephalopod species that also has multiple spawning cycles and a lifespan exceeding 20 years (Dunstan *et al.* 2011), are four times higher than those in *V. infernalis* (Baldwin 1982, Seibel *et al.* 1998). If the metabolic rate is a proxy for lifespan and growth, it sure seems that deep-sea pelagic octopodiforms are among the few cephalopod species able to surpass the typical 3 years lifespans.

The periodicity of the growth increment formation in beaks of *J. diaphana* and *V. infernalis* remains unknown, but their metabolic rates, reduced locomotory capabilities, feeding habits and reproductive strategy provide indirect evidence that the increments might require longer than one day to be deposited. Although the juveniles of both species may inhabit upper ocean layers, which are subject to night and day light regimes, most of the species' life cycles are spend at meso- and bathypelagic depths. The abilities of *Japetella* and *Vampyroteuthis* to inhabit oxygen minimum zones may originate from a slow pace of life history strategy and the consumption of readily available detrital material and zooplankton (Hoving *et al.* 2012, Gloeckler *et al.* 2018). The caloric intake from this low energy food source may not be sufficient to enable the fast growth rate necessary to reach maturity within one year, as suggested from the number of increments in the beaks.

Specimens of *J. diaphana* and *V. infernalis* have been successfully kept alive for some periods of time on board of research vessels or in laboratory aquaria (Seibel *et al.* 1999, Birk *et al.* 2019). The periodicity of growth increments deposition in the beaks of both species might be investigated by injecting the animals with fluorescent chemical markers, which get incorporated in the beaks and allow quantification of increment formation, a technique that has been applied for benthic octopod species (Perales-Raya *et al.* 2014). An alternative and novel non-invasive technique would be the use of ultrasound to monitor beak growth during a certain time interval and to correlate the interval with the number of growth increments after the animals' death. Ultrasound has been successfully used to assess sex and reproductive physiology in fishes (Næve *et al.* 2018) and might be also useful in cephalopod research.

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Authors' contributions

R.S. and H.J.H. designed the study; B.H.R. and H.J. collected specimens and beaks; R.S. processed specimens, reproductive systems, prepared and examined beaks for age estimation and wrote the manuscript draft; R.S. and V.L. measured eggs and estimated fecundity of J. diaphana; All authors are preparing the manuscript for submission to a peer reviewed journal.

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General discussion and outlook

Overall in this thesis I investigated the life histories of the Antarctic incirrate benthic octopods, of the deep-sea pelagic octopus *Japetella diaphana* and of the vampire squid *Vampyroteuthis infernalis*. In the first chapter, literature data of 26 octopod species were compiled and provided novel insights into the relationship between life history traits and environmental conditions. A major question was addressed: Are octopod lifespans inversely correlated with habitat temperature? As observed in other marine poikilotherms, low water temperatures can strongly delay embryonic development periods, reduce growth rates and increase the time to reach maturity, and hence extend lifespan. These traits, associated with the fact that all female incirrate octopods brood their eggs until the end of the embryonic development, can be used as an indirect tool to estimate longevity in this animal group. The estimates for time to reach maturity in Antarctic and deep-sea octopod species derived from embryonic development time at low temperatures still need further validation. However, the plot showing a negative allometric trend between time to reach maturity and temperature (chapter 1, Fig 3), was almost identical in shape to that observed for embryonic development of gastropods (Peck *et al*. 2006a) and bivalve molluscs (Peck *et al*. 2007) from a wide latitudinal range.

Thorson's rule (Mileikovsky 1971), which states that egg size and embryonic development in benthic poikilotherms are positively correlated with latitude has been tested and confirmed for several taxa, including octopods (Ibáñez *et al*. 2018). The octopod lineage containing Antarctic and deep-sea species seems to have evolved to produce large eggs that require extended embryonic development but will result in benthic crawling hatchlings, which can avoid the high mortality rates experienced by planktotrophic larvae commonly produced by temperate species (Peck *et al*. 2006b, Strugnell *et al*. 2008, Ibáñez *et al*. 2018). As female octopods brooding eggs are known to cease feeding, they may also require a considerable amount of time before attaining body sizes that can be used as energy resource (i.e. tissue breakdown) until the embryos hatch. Females of deep-sea octopod species brooding eggs in temperatures of 2 to 7°C have been reported to survive extended periods of time from 13 to 53 months (Wood *et al*. 1998, Robison *et al*. 2014). Therefore, the estimates presented in my thesis suggest that Antarctic and deep-sea octopod

species may require 3 to 5 years to reach maturity and have lifespans exceeding 8 years seem to be a reasonable approximation.

The lifespan estimates for cold-water octopods based on water temperature and life history traits were further investigated in the second chapter, by means of quantification of growth increments deposited in the chitinous beaks of Antarctic species. The assumption was that the beaks of Antarctic octopods would have a higher number of growth increments than observed in temperate and tropical species. Age estimates for mature specimens of temperate and tropical *Octopus* species (*O*. *maya* and *O*. *vulgaris*) in which beak increments had been validated to be deposited daily ranged from 200 to 734 days (Rodríguez-Domínguez *et al*. 2013, Perales-Raya *et al*. 2014). The present results show that species of the Antarctic octopod genus *Pareledone* which mature at small sizes of less than 100 g (e.g., *P*. *aequipapillae* and *P*. *charcoti*) had in average 296 to 433 growth increments in their beaks, while submature specimens of the large species *Megaleledone setebos* had between 700-800 increments. However, the temperate and tropical *Octopus* species live in average habitat temperatures of 22-25°C while the Antarctic octopod species experience temperatures of -2 to 2° C. If the physiological processes in Antarctic octopod species experience the same temperature effects (Q_{10}) observed in temperatures between 5° and 30°C, growth increments might be deposited at a rate that is 2-4 fold slower than in their temperate counterparts. The Arrhenius relationship fitted for octopods in the first chapter of my thesis shows that Q_{10} for species living below 5°C can be even higher than 4.

This assumption suggests that *Pareledone* species become mature after 3 to 5 years and *Megaleledone setebos* after 8 years. Considering that the females need to brood large eggs of 10 to 40 mm in size in water temperatures near or below 0°C, the total lifespan in these species may reach 8 to 12 years. A few *Pareledone charcoti* females that had been kept in aquaria for ~12 months were still immature and had between 219-364 growth increments in their beaks. This observation provides further evidence that increments require more than one day to be deposited. Moreover, the anecdotal record of a *P*. *charcoti* specimen that was kept alive for about eight years at the Alfred Wegener Institute (Dr. Felix Mark, pers. comm.) confirms that the species can achieve lifespans estimated by the indirect method applied in the thesis.

The present Antarctic octopod dataset represents a snapshot of the population biology of the species inhabiting the Antarctic Peninsula and the South Shetland Islands in the Atlantic sector of the Southern Ocean. The samples were restricted to autumn months (March and April 2012), but a wide range of body sizes and maturity stages were present in all species. This suggests that the spawning period is extended or occurs throughout the whole year as observed in former studies on octopod species from South Georgia Island and the Antarctic Peninsula (Kuehl 1988, Yau *et al*. 2002, Barratt *et al*. 2008). This also suggests a complex population structure, with animals representing potentially different annual cohorts with reproduction and recruitment happening throughout the year. Sampling limitations restricted to the summer/autumn periods makes it difficult to further investigate these assumptions. Many aspects of the life histories of the Southern Ocean octopod fauna such as feeding habits, embryonic development, hatchling sizes and trophic position remain poorly known and require further research.

The third chapter of my thesis aimed to assess if the deep-sea pelagic octopodiforms *Japetella diaphana* and *Vampyroteuthis infernalis* have longer lifespans compared to benthic relatives. Age estimates using growth increments quantified in the beaks of the two species, and assuming a daily periodicity, suggest that growth rates and lifespans may not be too dissimilar to those species from tropical and temperate regions. However, these results are incongruent with the reproductive strategies, feeding habits and metabolic rates of these species that suggest long lives and very slow growth rates. Metabolic rates measured for *J. diaphana* and *V. infernalis* are lower than those of *Nautilus pompilius* and *Pareledone charcoti* (Baldwin 1982, Seibel *et al*. 1998, Seibel 2007), cephalopods that have been observed to live longer than 8 years while in captivity (Dunstan *et al*. 2011).

The most mature female vampire squid examined in the third chapter had a female with a body weight of 138 g and showed 375 growth increments in its beak. The reproductive strategy of vampire squid suggests that animals can continue to grow during the gonadal resting phase between reproductive cycles. Although it is premature to assume that the mature female mentioned above would continue to grow and reproduce, many museum specimens in a similar reproductive condition weighed between 300-500 g. As metabolic rates are inversely correlated with body mass, large females feeding on low energy particulate organic matter may require a

considerable time to grow in the low temperatures of the bathypelagic zone in the deep-sea.

The upper beaks of the brooding female of *J. diaphana* had between 192-197 increments. Brooding females were always observed at depths between 900 and 1500 m where water temperatures were below 4°C (Young 1972, 1978; thesis chapter 3). At this temperature, embryonic development may require as much as two years. It is possible that some females move to shallower and warmer waters to speed up embryonic development, since hatchlings are typically found in the upper limit of the species depth distribution. If this is the case, the females must migrate to shallower depths only at the end of the embryonic development to avoid the predation risks associated with this behavior. The growth-increment deposition rates in the beaks of *J. diaphana* and *V. infernalis* remain unknown, but as well as observed in the Antarctic octopod species, indirect evidence suggests that increments require more than one day to be formed.

Future perspectives

The development of new culture techniques may help to improve our understanding of octopod life histories in general. Many species do well under laboratory conditions, and animals of known age can help to develop better models to estimate age in wild animals. The model used to estimate age at maturity based on embryonic development and average temperatures in the first chapter of this thesis can be improved incorporating metabolic and food conversion rates. Although there are limitations for keeping deep-sea pelagic octopodiforms under laboratory conditions (e.g., light, temperature), Antarctic and deep-sea benthic octopods have been kept in captivity for extended periods of time. The administration of oxytetracycline and the injection of calcofluor in husbandry experiments proved to be successful age validation methods in stylets and beaks, respectively and should also be suitable for experiments on cold-water benthic octopods.

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Appendix

Supplement S1 – Chapter 1

Life history traits table references

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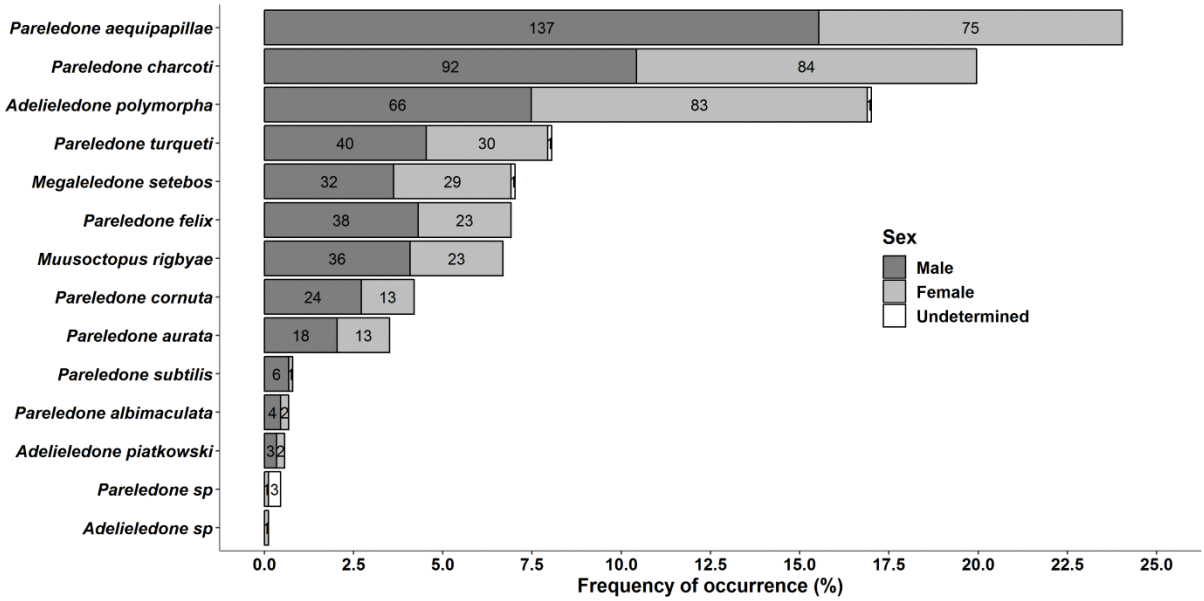
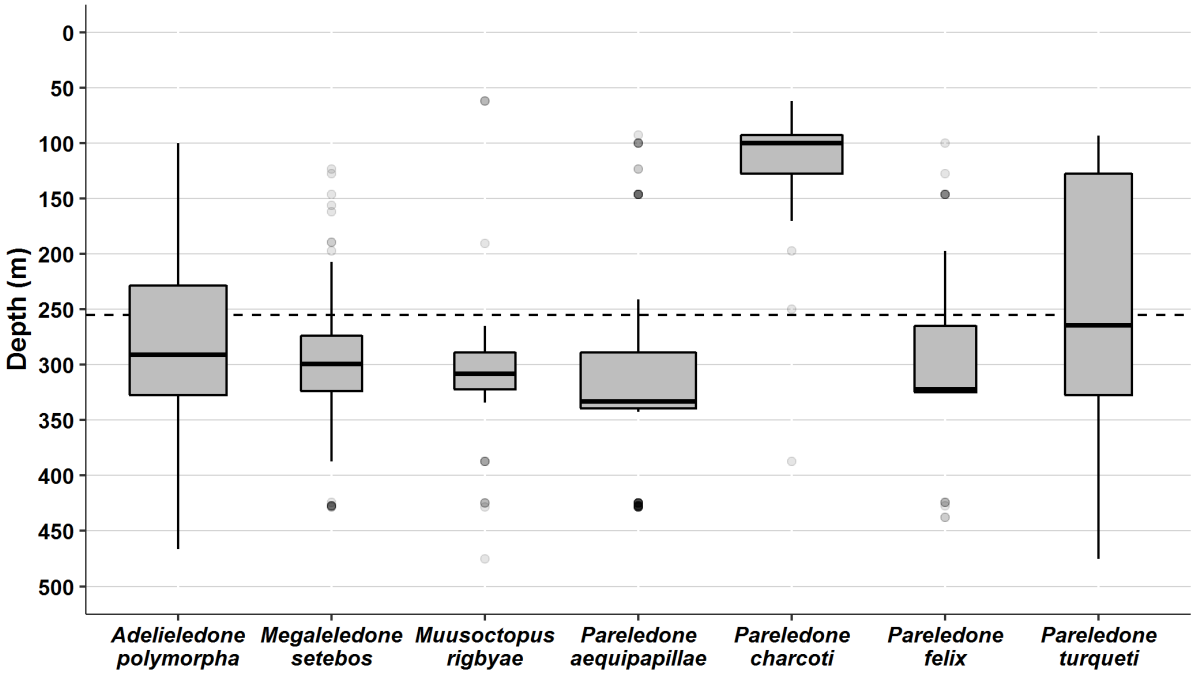
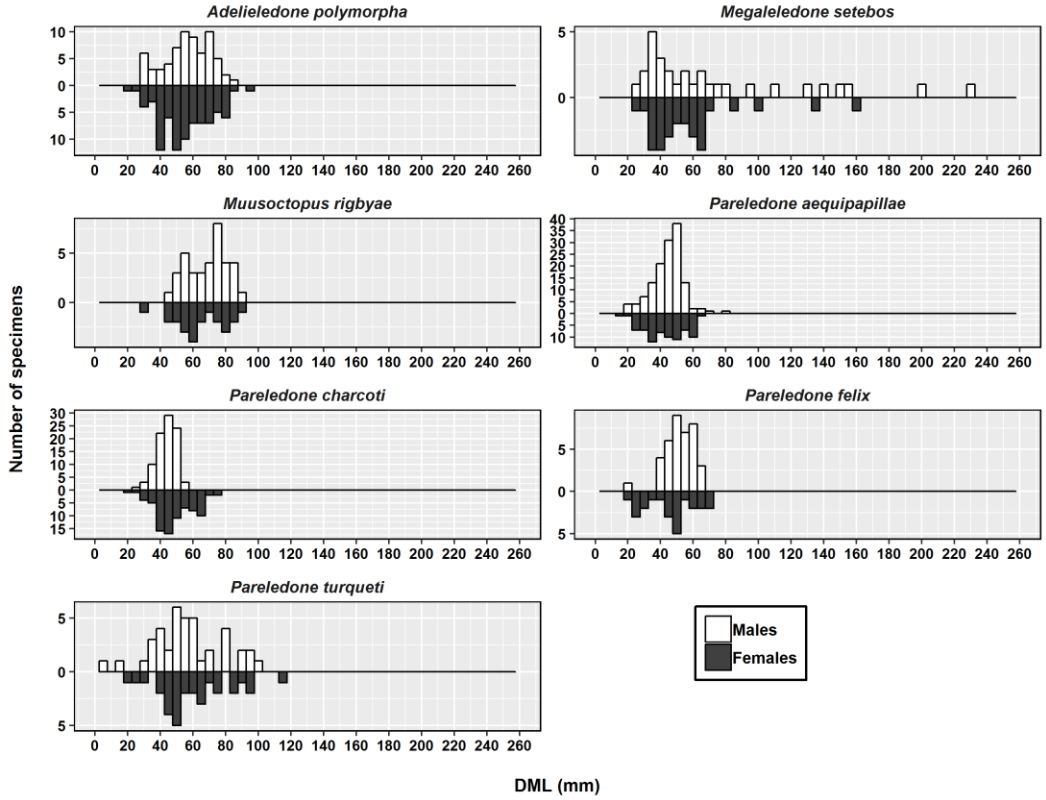
Supplement S2 – Chapter 2



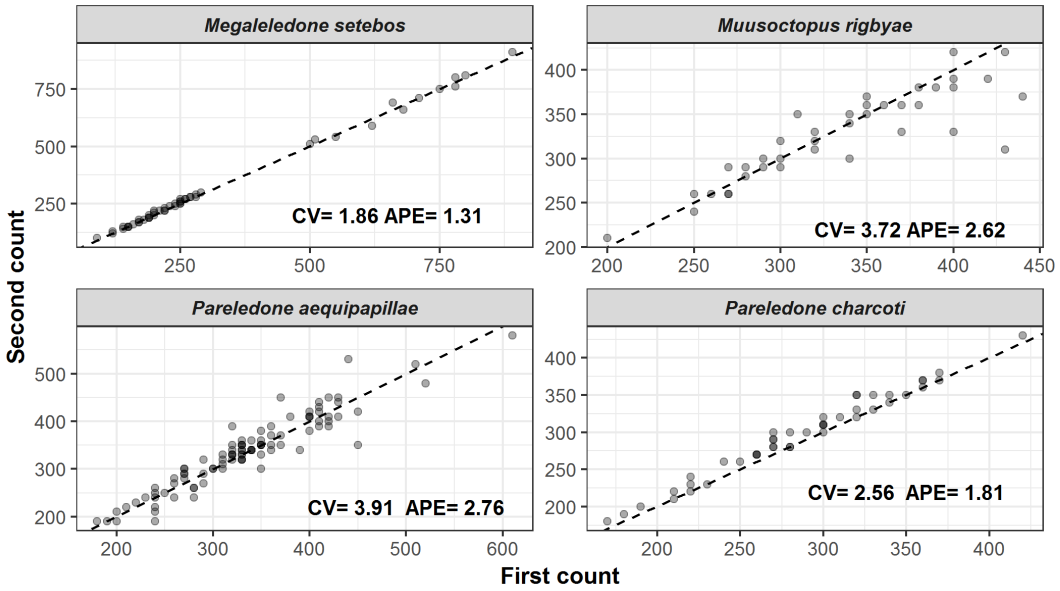
S2 Fig 1. Frequency of occurrence of the Antarctic octopod species analyzed. Values inside bars represent the number of animals examined.



S2 Fig 2. Depth distribution of the most numerous species (N > 50) captured during the RV POLARSTERN cruise PS79 in 2012. The dotted line represents the average depth of the bottom trawls ($\bar{x}$ = 255 m). The horizontal black lines inside boxes refer to the median; boxes and whiskers extend from the 25th to the 75th percentile; circles represent outlier cases.



S2 Fig 3. Frequency distribution of dorsal mantle lengths of males and females of the most numerous species analyzed.



S2 Fig 4. Bias plot comparing the precision of two counts of growth increments in the upper beak lateral walls. The dashed line represents the match between the two counts. The coefficient of variation (CV) and average percent error (APE) are presented for each species.

S2 Table 1. Estimated model parameters of the relationship between mantle length and body mass for seven Antarctic octopod species. The equation is given as BM = aDML^b, where BM - body mass (g) and DML - dorsal mantle length (mm). Standard error in parentheses. ♂ - male; ♀ - female.

Species	*a*	*b*	*p* value	R^2	N
Adelieledone polymorpha	1.15×10^{-2} (0.01)	2.12 (0.11)	< 0.001	0.77	**149**
♂	6.34×10^{-3} (0.01)	2.24 (0.23)	< 0.001	0.80	66
♀	7.01×10^{-3} (0.00)	2.23 (0.16)	< 0.001	0.75	83
Megaleledone setebos	1.39×10^{-4} (0.00)	3.32 (0.07)	< 0.001	0.98	**59**
♂	9.89×10^{-7} (0.00)	4.23 (0.16)	< 0.001	0.97	30
♀	2.91×10^{-4} (0.00)	3.17 (0.04)	< 0.001	0.98	29
Muusoctopus rigbyae	4.29×10^{-3} (0.01)	2.44 (0.29)	< 0.001	0.63	**59**
♂	9.32×10^{-4} (0.00)	2.79 (0.45)	< 0.001	0.65	36
♀	5.91×10^{-3} (0.01)	2.37 (0.43)	< 0.001	0.62	23
Pareledone aequipapillae	1.48×10^{-2} (0.01)	2.01 (0.09)	< 0.001	0.74	**212**
♂	1.83×10^{-2} (0.01)	1.95 (0.12)	< 0.001	0.67	137
♀	1.04×10^{-2} (0.01)	2.11 (0.16)	< 0.001	0.83	75
Pareledone charcoti	2.61×10^{-3} (0.01)	2.19 (0.20)	< 0.001	0.80	**176**
♂	4.55×10^{-2} (0.04)	1.69 (0.24)	< 0.001	0.54	92
♀	4.75×10^{-3} (0.00)	2.33 (0.09)	< 0.001	0.83	84
Pareledone felix	7.54×10^{-3} (0.01)	2.19 (0.20)	< 0.001	0.76	**61**
♂	1.22×10^{-2} (0.02)	2.05 (0.33)	< 0.001	0.61	38
♀	3.26×10^{-3} (0.00)	2.41 (0.28)	< 0.001	0.88	23
Pareledone turqueti	9.40×10^{-4} (0.00)	2.81 (0.19)	< 0.001	0.82	**70**
♂	4.01×10^{-4} (0.00)	3.01 (0.37)	< 0.001	0.75	40
♀	5.75×10^{-4} (0.00)	2.89 (0.33)	< 0.001	0.94	30

S2 Table 2. Estimated model parameters of the relationship between growth-increments number in upper beaks and body mass for four Antarctic octopod species. The equation is given as $BM = a ginc^b$, where BM - body mass (g) and g*inc* – growth increments. Standard error in parentheses.

Species	*a*	*b*	*p* value	R^2	N
Megaleledone setebos	9.95×10^{-10} (0.00)	4.34 (0.44)	< 0.001	0.94	50
Muusoctopus rigbyae	8.38×10^{-5} (0.00)	2.41 (0.41)	< 0.001	0.58	38
Pareledone aequipapillae	6.45×10^{-3} (0.01)	1.45 (0.15)	< 0.001	0.69	133
Pareledone charcoti	1.43×10^{-5} (0.00)	2.59 (0.53)	< 0.001	0.46	44

Supplement S3 – Chapter 3

Table S3. Estimated model parameters of the relationship between mantle length and body mass for *Japetella diaphana* and *Vampyroteuthis infernalis*. The equation is given as $BM = aML^b$, where BM - body mass (g) and ML - dorsal mantle length (mm). Standard error in parentheses.

Species	*a*	*b*	*p*	R^2	N	DML
J. diaphana	9.97×10^{-5} (0.0001)	2.98 (0.13)	< 0.001	0.87	37	3 - 73
V. infernalis	1.19×10^{-4} (0.0009)	2.99 (0.12)	< 0.001	0.71	50	12 - 136

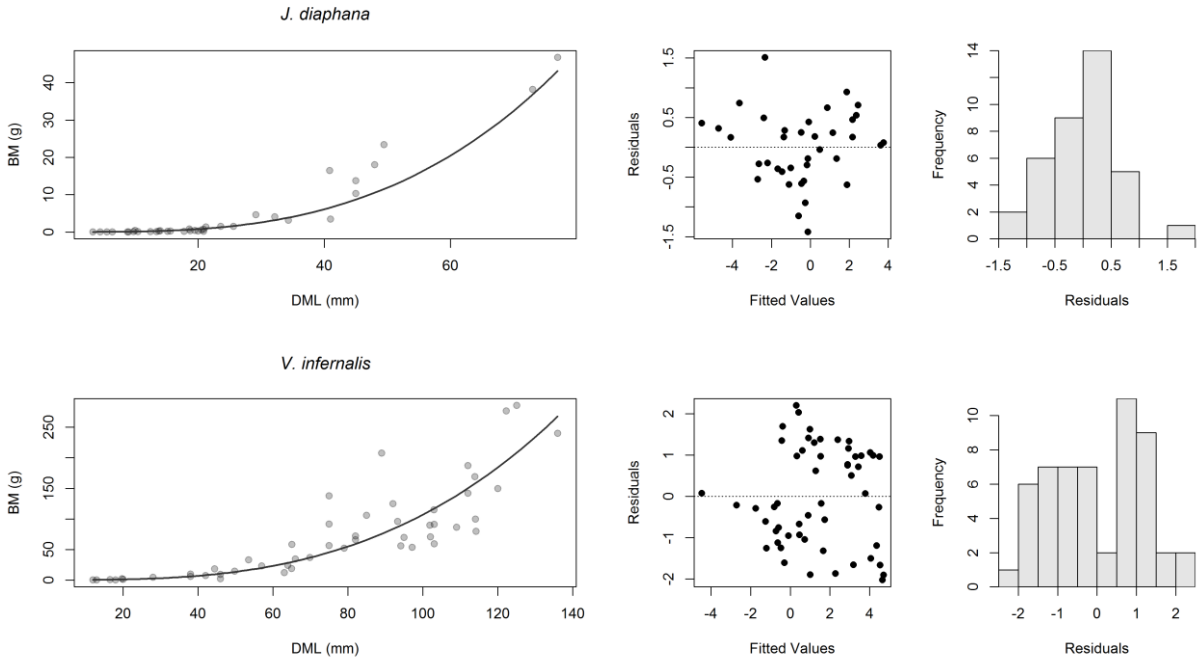


Fig S3. Relationship between dorsal mantle length and body weight for thawed specimens of *J. diaphana* and *V. infernalis*. Solid line represents the best, dashed lines are confidence intervals (2.5%-97.5%). Parameter estimates are in Table S3.

Author contributions

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Manuscript 1: Chapter 1

“Impact of environmental temperature on the lifespan of octopods”

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Contributions: R.S., H.J.H and U.P. designed the study; R.S. collected the data, performed the analyses and wrote the first manuscript draft. All authors edited and revised the manuscript.

Manuscript 2: Chapter 2

“Life histories of Antarctic incirrate octopods (Cephalopoda: Octopoda)”

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Contributions: R.S., H.J.H. and U.P. designed the study; C.N. collected octopods during the Polarstern 2012 expedition to the Southern Ocean; R.S. processed the specimens, collected, prepared and processed the beaks for age estimation, extracted and analyzed reproductive systems and wrote the first manuscript draft; All authors wrote and edited the manuscript for journal submission.

Manuscript 3: Chapter 3

“Quantification of beak increments to study the pace of life in pelagic deep-sea Octopodiformes *Japetella diaphana* and *Vampyroteuthis infernalis*”

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Status: Manuscript in preparation

R.S. and H.J.H. designed the study; B.H.R. and H.J. collected specimens and beaks; R.S. processed specimens, reproductive systems, prepared and examined beaks for age estimation and wrote the manuscript draft; R.S. and V.L. measured eggs and estimated fecundity of J. diaphana; All authors are preparing the manuscript for submission to a peer reviewed journal.

Eidesstattliche Erklärung

Hiermit erkläre ich, dass ich die von mir vorgelegte Dissertation, bis auf die Beratung durch meine Betreuer, selbstständig und ohne Zuhilfenahme unerlaubter Hilfsmittel angefertigt habe.

Alle benutzten Quellen und Hilfsmittel habe ich vollständig angegeben und die Zusammenarbeit mit anderen Wissenschaftlern habe ich kenntlich gemacht. Zudem versichere ich, dass diese Arbeit unter Einhaltung der Regeln guter wissenschaftlicher Praxis der Deutschen Forschungsgesellschaft verfasst wurde, und dass sie nach Form und Inhalt meine eigene Arbeit ist. Weder diese noch eine ähnliche Arbeit wurden an einer anderen Abteilung oder Hochschule im Rahmen eines Prüfungsverfahrens vorgelegt, veröffentlicht oder zur Veröffentlichung vorgelegt. Dies ist mein erstes und einziges Promotionsverfahren. Mir wurde kein akademischer Grad entzogen.

Kiel, 17.06.2019

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Richard Schwarz

Eidesstattliche Erklärung

Declaration

I, Richard Schwarz, hereby declare that the dissertation submitted, entitled "**Assessing the lifespans of coldwater octopods (Cephalopoda: Octopodiformes)**" was written independently by me and only using the sources listed. The content and design of this thesis, apart from the supervisor's guidance, is my own work. The thesis has not been submitted either partially or wholly as a part of a doctoral degree to another examining body and is my first and only doctoral procedure. Chapter 1 of this thesis was published in a peer reviewed journal in 2018, while chapter 2 has been resubmitted to a peer reviewed journal after revisions and is currently under review. The third chapter will be submitted to a peer-review journal. The contributions to the manuscripts made by myself and my co-authors are explained in the section "Author contributions". This work has been respecting the Rules of Good Scientific Practice of The German Research Foundation. I have not been deprived of an academic degree.

Kiel, 17.06.2019

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Richard Schwarz

Curriculum Vitae

Richard Schwarz

Birth date and place: 28.12.1983 Chapecó, Santa Catarina, Brazil.

Research interests: Oceanography, fisheries research, statistics, population ecology, cephalopods, deep-sea, sclerochronology.

Language skills: Portuguese - native speaker; English - good command / proficient in spoken and written English (TOEFL); Spanish - basic communication skills; German - basic communication skills.

Formal Education:

2015-2019

PhD Candidate at GEOMAR/ Christian-Albrechts-Universität zu Kiel, Germany.

Thesis title: Assessing the lifespans of coldwater octopods (Cephalopoda: Octopodiformes)

Supervisor: Dr. Henk-Jan Hoving
Co-supervisor (mentor): Dr. Uwe Piatkowski
Scholarship from: CNPq, Brazil, grant: CNPq/GDE (201585/2015-4) – 45 months.

2007 - 2009

Master degree in Environmental Science and Technology (multidisciplinary)
Universidade do Vale do Itajaí, UNIVALI, Itajai, Brazil

Title of dissertation: Utilização da microestrutura do estatólito como ferramenta na avaliação dos padrões populacionais do Calamar argentino *Illex argentinus* (CEPHALOPODA: OMMASTREPHIDAE) relevantes a gestão da pesca de talude no sudeste e sul do Brasil

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2002 - 2006
Bachelor in Oceanography
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Supervisor: José Angel Alvarez Perez

Peer reviewed publications

Schwarz R, Hoving HJ, Noever C, Piatkowski U (2019) Life histories of Antarctic incirrate octopods (Cephalopoda: Octopoda). PLOS ONE 14(7): e0219694. https://doi.org/10.1371/journal.pone.0219694

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Schwarz R, Perez JAA (2007) Diferenciação populacional do calamar argentino *Illex argentinus* (Cephalopoda: Teuthidae) no sul do Brasil através da morfologia e morfometria do estatólito. Brazilian Journal of Aquatic Science and Technology (*In Portuguese*), 11, 1-12.